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IN VIVO AND *IN SITU* INVESTIGATIVE RESULTS OF REPAIR AND RECOVERY PROCESSES DURING ONTOGENESIS, AFTER X-RAY IRRADIATION OF BEAN SEEDS

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When exposing plant organs to high doses of ionizing radiation, disorders in growth and development or even lethality may occur. With the aim of modelling this phenomenon, seeds of bean, variety Echo Elit, were irradiated with a 300 Gy dose of X-ray irradiation (120 kV; 4.5 mA). In order to characterize repair and recovery at plant level, the biological production and photosynthetic pigments of the plants during ontogenesis *in vivo* and changes in their electric capacitance were continuously monitored and recorded *via* a computer-aided and -controlled data acquisition system. According to the data obtained, the repair in the biosynthesis of photosynthetic pigments will have been completed by the beginning of flowering. It may be assumed from the capacitance measurements that the recovery from radiation injuries can be traced by such measurements, while the repair process can be identified by relating the capacitance values to measurements made at 11 a.m. According to this postulation the process of repairing X-ray injuries might be finished by the beginning of pod formation without the plants actually recovering.

Key words: X-ray irradiation; *Phaseolus vulgaris* L., photosynthetic pigments, capacitance; repair

Abbreviations: B, budding; BF, beginning of flowering; BPF, beginning of pod formation; total carotenoids: xanthophylls and carotenes; chl a, chlorophyll a; chl b, chlorophyll b; nF, nanofaraday; pF picofaraday; kV, kilovolt; mA, milliampère; photosynthetic pigments: chlorophyll a + b + xanthophylls + carotenes

Introduction

After treating plant organs, tissues and cells with ionizing irradiation, physical, physicochemical, chemical, biochemical and physiological chain effects occur (Kőrösi and Pál, 1987).

For these processes the basic questions asked concern the repair and consequent recovery at various levels (e.g. molecular, gene, chromosome, biochemical, morphological, biological production) and their manifestation during ontogenesis (Tobias, 1985; Weber, 1988; Kőrösi and Pál, 1989; Bushkyavichus, 1990; Shirley et al., 1992).

Sequence analysis of the break points in chalcone isomerase and dihydroflavonol-4 reductates of two *Arabidopsis* transparent testa mutations indicated that the repair of radiation-induced damage involved mechanisms similar to those that mediate the integration of foreign sequences into the genome (Shirley et al., 1992).

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The three-fold fractionation of the 45 Gy X-ray dose delivered to *Gerbera jamesonii* showed a change in the marker gene (dark centre of the flower) (Walther and Sauer, 1991).

Mitotic aberrations in the root tips, anaphase bridges and micronuclei at telophase were considerably fewer in number when GA_3 treatment followed gamma-irradiation. The results suggest that GA_3 probably reduced the cytological damage by preventing the potential damage from becoming actually detectable, thereby promoting the repair process (Arora et al., 1989). Other authors also confirm the hormonal background of changes in the metabolism of irradiated plants (Sax, 1963; Kryukova, 1975; Kuzin and Vagabova, 1981; Jezierska-Szabó et al., 1986; Zubko and Zubko, 1994; Melyan, 1994; Al-Bachir, 1995). In consequence, an alteration in correlative development and growth may occur (Kőrösi and Pál, 1989).

At the plant level, recovery from radiation injuries in line A26 of *Gerbera jamesonii* was characterized by the number of shoots developing during a period of 16 weeks, measured at 4 subsequent dates of cutoff, as well as by propagation profiles, and by calculating a recovery factor (Walther and Sauer, 1990).

In the present work an *in vivo* method was developed to follow the recovery of the bean plants during ontogenesis. *In situ* alterations in the electric capacitance were registered during ontogenesis. To relate data gained in this way to the repair and recovery of radiation-injured plants, the biological production, defined as dry matter, and the quantity of photosynthetic pigments were measured and used as basic parameters.

Material and methods

Plant material

Air-dried bean seeds (water content 9.3%), of the licensed variety Echo Elit were used, with a thousand seed weight of 186.5 g. The seeds were grown in pots. Ten replications were used for the treatment and control, with three plants per pot. The pots were filled with earthworm humus in order to ensure proper plant growth and development. The plants were grown in a growth chamber at a temperature of 24 ± 2°C, with a 14/10 hour light/dark photoperiod and a light intensity of 85.45 $\mu E/s/m^2$ at 50 cm from the surface. The air humidity was adjusted to 70%. To assess the biological production of the plants, the aboveground organs (stem, leaves and pods) were separated and their dry matter was measured.

The confidence limits of the mean, and standard deviations were used for the statistical evaluation of the data (Mendenhall, 1987).

Irradiation treatment

The X-ray irradiation was carried out using Liliput 140 X-ray emitting apparatus (Medicor, Hungary) at the Central Laboratory of the Agricultural University of Gödöllő. The X-ray apparatus was set to 4.5 mA at 120 kV.

According to preliminary studies, a dose of 300 Gy, under the technical conditions stated above, caused injuries to the exposed seed that could be repaired during ontogenesis. Therefore, in the present work 300 Gy doses were applied.

Determination of chlorophyll-a, -b and carotenoids

In order to monitor the recovery of the photosynthetic pigment-synthesizing capacity of radiation-injured plants the photosynthetic pigments (chl a, chl b and the total carotenoids) were quantitatively determined, using 100% acetone for extraction. The pigment concentrations were determined at three phenological stages: budding, flowering and the beginning of pod formation. According to the final chl content, 100–200 mg of fresh plant leaves were weighed, transferred to a chilled mortar and homogenized by adding approx. 0.1 g $CaCO_3$. The extraction from the homogenized sample was performed with chilled 100% acetone, using a total of 15 ml of acetone, in several steps. Each portion of the extract was transferred to a centrifuge tube and the extract was centrifuged at 3000 rpm for 5 minutes. The supernatant was then transferred to a chilled tube, the final volume of which was adjusted to 20 ml with chilled 100% acetone. After this the absorption was recorded for chlorophyll a and b and carotenoids at 662 nm, 644 nm and 440.5 nm, respectively. All the operations described were accomplished at low light intensity and as rapidly as possible (e.g. extraction within 3 minutes).

The optical density of the extracted solutes was measured at wave-lengths of 662 nm, 644 nm and 440.5 nm for chlorophyll a, b and carotenoids, respectively, utilizing a Zeiss Spectrophotometer Spekol-1, Jena, Germany. For calculations of pigment contents, extinction coefficients from Lichtenthaler (1987) were applied and were related to dry matter.

Measuring of the capacitance of the plants

The bioelectrical properties of the plants consist of an active and a passive component. The active characteristic results from the metabolism responding to various environmental stimuli, while the passive one is coupled to the changes in the plant tissues. The dielectric features of plants (e.g. permittivity, phase of impedance, capacitance) may outline the physical state of the plants *in vivo* (László and Kristóf, 1982; László, 1982a, 1982b, 1983). For the measurements platinum needle electrodes were connected to the stems at defined points. After a 5–7 day period the mechanically injured tissues formed calli and the connections within the vascular system were restored. Capacitance measurements with data acquisition during ontogenesis were controlled and carried out by a PC computer every twenty minutes. This on line method ensured both the reliability of the measurements and accurate data processing. According to preliminary studies on the circadian rhythm of the capacitance changes (pF), the data taken into consideration were those from 11 a.m. to 2 p.m., with the 11 a.m. values being the base the alterations were related to.

Results

Growth and biological production of bean developed from X-ray-irradiated seeds

As seen from Table 1, 300 Gy X-ray irradiation retarded the emergence of beans by more than 40%. This meant that a severe hampering effect occurred, which had to be repaired and restored during ontogenesis.

The aboveground biological production of surviving plants was restored by the beginning of pod formation, even resulting in a surplus (Table 2).

Table 1

Effect of 300 Gy X-ray irradiation on the emergence percentage of the bean variety Echo Elit

Treatment	Average (%)	Confidence interval (p=5%)	As a % of the control
Non-irradiated	85.15	67.25–100.02	100.00
300 Gy	49.79	32.11– 67.47	58.47

Table 2
Aboveground biological production of surviving plants after repair from 300 Gy X-ray irradiation

Treatment	Dry matter (g/plant)	Confidence interval (p=5%)	As a % of the control
Non-irradiated	2.74	2.55–2.93	100.00
300 Gy	3.75	3.10–4.39	136.86

Manifestation of repair in the biosynthesis of photosynthetic pigments during ontogenesis

In plants developed from seeds irradiated with 300 Gy, various chl deficiency symptoms were observed. Frequently encountered were white spots on the leaf, and leaf sectors showing light green colours. As the plants developed, the severity of the deficiency symptoms gradually lessened.

So as to monitor the repair of the biosynthesis of photosynthetic pigments, the chl a, b and carotenoids concentrations were determined at three phenological stages. It is clear from the data presented in Table 3 that during the period commencing at the beginning of budding and lasting till the beginning of pod formation, the chlorophyll-a, -b and carotenoids synthesizing capacity of the leaves was repaired and restored. Later, from flowering until pod formation, an overcompensation occurred. This resulted in an increase of more than 10% in the total pigment concentration. However, it should be noted that during the repairing and overcompensation period the chlorophyll-a/b and chlorophyll-(a + b)/carotenoids contents did not change significantly.

Effect of X-ray irradiation on electrolytic capacitance patterns in bean plants

When changes in the electrolytic capacitance of non-irradiated and irradiated plants were compared, it could be stated that the irradiated plants responded with significantly higher capacitance values and amplitudes during the period investigated (from budding to pod formation) (Fig. 1). The differences in the amplitudes of the capacitance, related to the basic 11 a.m. measurements, showed the highest alterations in the course of flowering (Fig. 2). However, these differences, as witnessed in Figure 2, lessened and disappeared during pod formation.

Discussion

From the point of view of plant stress and environmental physiology it is important to determine to what extent plants are capable of repairing primary lesions that cause injuries in the macromolecules during energy deposition along the track structures of primary and secondary ionizing particles.

In the present experiment, in order to model and bring about severe radiation injury, bean seeds were exposed to an X-ray dose of 300 Gy. This

Table 3
Changes in the pigment concentrations in bean leaves during repair

Photosynthetic pigments ($mg \times g[DW]^{-1}$)	Pheno-phase	Non-irradiated		Irradiated		
		Average	Confidence interval (p=5%)	Average	Confidence interval (p=5%)	As a % of the control
Chl a	B	15.23	14.12–16.33	10.36	8.66–13.06	68.02
	BF	16.48	14.50–18.45	15.10	12.06–18.13	91.62
	BPF	10.89	8.84–12.94	11.81	10.07–13.53	108.45
Chl b	B	4.45	4.20–4.71	3.17	2.60–3.74	71.23
	BF	5.75	4.90–6.59	5.54	4.27–6.81	96.35
Chl a + b	BPF	4.09	3.47–4.71	4.71	3.93–5.48	115.16
	B	19.69	18.37–20.99	13.54	11.27–15.80	68.77
	BF	22.24	19.41–25.05	20.65	16.34–24.94	92.85
	BPF	14.99	12.32–17.65	16.52	14.03–18.99	110.21
Carotenoids	B	4.30	3.91–4.67	3.00	2.57–3.42	69.76
	BF	5.26	4.84–5.66	4.63	3.92–5.34	88.02
	BPF	3.77	3.23–4.30	4.15	3.46–4.84	110.08
Chl (a+b+car)	B	23.98	22.33–25.63	16.54	13.86–19.22	68.97
	BF	27.49	24.28–30.69	25.28	20.27–30.27	91.96
	BPF	18.76	15.57–21.94	20.81	17.63–23.99	110.93
Chl a/b	B	3.42	3.25–3.57	3.29	3.20–3.36	96.19
	BF	2.87	2.78–2.95	2.73	2.60–2.85	95.12
	BPF	2.65	2.54–2.74	2.52	2.38–2.66	95.09
Chlorophyll (a+b)/ carotenoids	B	4.61	4.40–4.80	4.49	4.29–4.67	97.40
	BF	4.23	3.97–4.47	4.44	4.17–4.71	104.96
	BPF	3.96	3.74–4.16	3.86	3.74–3.97	97.47

The pigment concentrations were determined at three phenological stages: B, budding; BF, beginning of flowering and BPF, beginning of pod formation

treatment reduced the emergence percentage by about 50%, compared to that of the control sample, which is considered to be a considerable hampering effect (Table 1). As evidenced through the biological production of surviving plants, the healing process resulted in a complete recovery by the beginning of pod formation (Table 2). This can possibly be related to alterations in the growth regulator status of the irradiated plants (Jezierska-Szabó et al., 1986; Lage and Esquibel, 1995). This relationship is also supported by findings showing that the impeding effect of irradiation was counteracted by exogenously applied plant growth regulating substances (Masahiro et al., 1974; Kryukova, 1975; Ahmad and Trifu, 1980; Bushkyavichus, 1990; Ben-Amer and Borner, 1994; Melyan, 1994).

In view of the present results, the repair may be related to the biosynthetic pathways of chlorophyll a, b and carotenoids, as is evidenced in Table 3. Regarding the photosynthetic pigment concentrations, a healing period in the synthesizing capacity of the pigments, lasting from the beginning of emergence to flowering, is postulated.

Experimental evidence was gained that the repair of bean plants from irradiation injuries can be described *in vivo* and followed by means of electrolytic capacitance measurements. Changes in the electrolytic capacitance of plants undergoing repair followed a pattern in which their capacitance values, in comparison to non-irradiated plants, were significantly higher on a time scale (ontogenesis) (Fig. 1).

When the capacitance values were related to the 11 a.m. measurement, a more distinct picture emerged concerning the reparation process. The differences determined showed a peak during flowering, which gradually declined during pod formation (Fig. 2).

It could be postulated from the capacitance measurements that the recovery can be traced using absolute capacitance measurements (Fig. 1), while repair can possibly be characterized by relating the capacitance values to the 11 a.m. measurements. According to this postulation, although the repair of X-ray injuries appeared to be finished by the beginning of pod formation (Fig. 2), the actual recovery of the plants did not take place (Fig. 1).

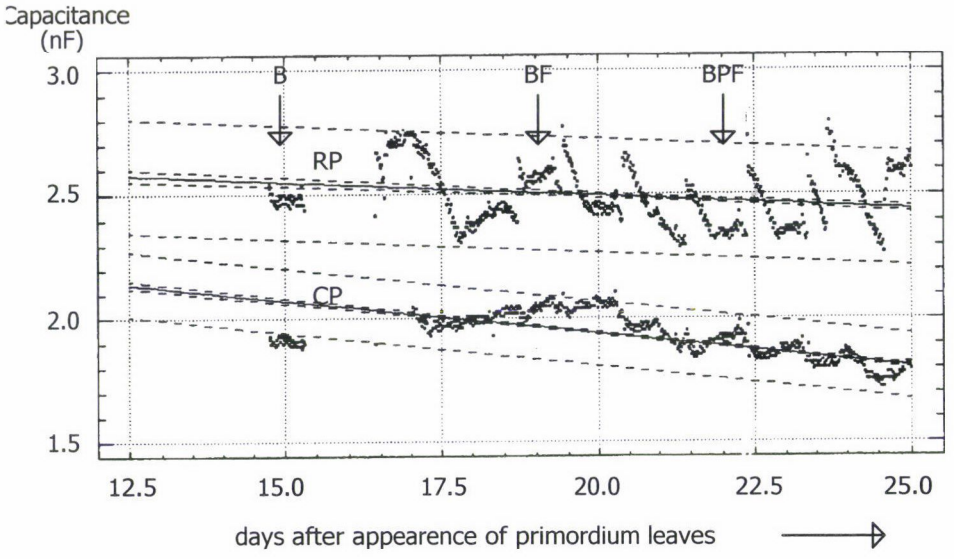

Fig. 1. Recovery of Echo Elit bean plants from X-ray injuries caused by 300 Gy (120 kV, 4.5 mA) seed irradiation as traced with capacitance measurements from budding to pod formation. IBM computer-controlled measurements and data acquisition were performed every 20 minutes each day, from 11 a.m. to 2 p.m. as described in the Material and methods. The values are averages of 10 repetitions±sd. B = budding; BF = beginning of flowering [≤ 25% of plants had flowers]; BPF = beginning of pod formation [≤ 25 % of plants began to develop pods]. RP = recovering plants; CP = control plants

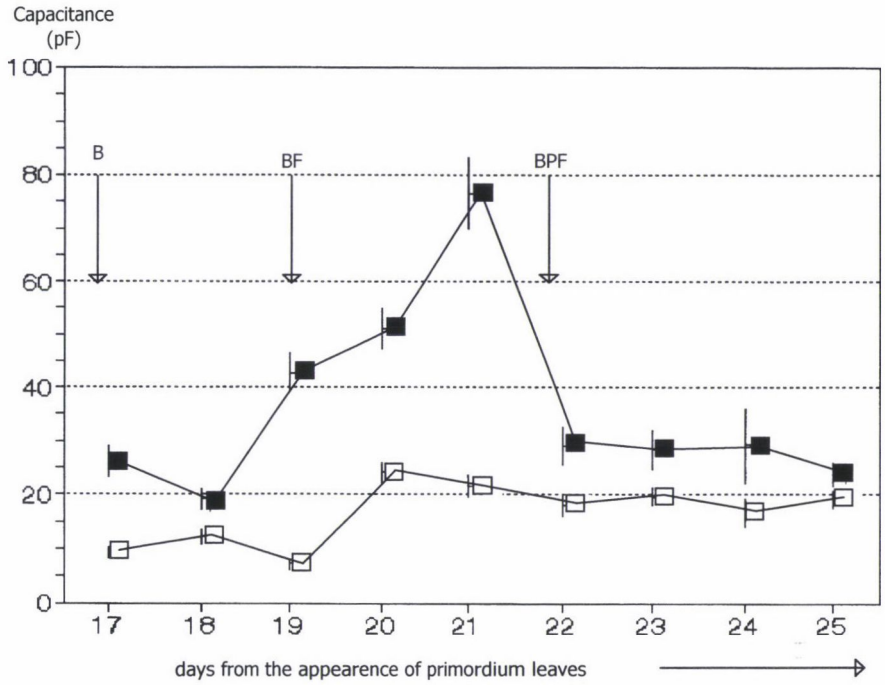

Fig. 2. Repair of Echo Elit bean plants from X-ray injuries caused by 300 Gy (120 kV, 4.5 mA) seed irradiation, as traced with capacitance measurements related to 11 a.m. values from budding to pod formation. IBM computer-controlled measurements and data acquisition were performed every 20 minutes each day, from 11 a.m. to 2 p.m. as described in the Material and methods. The values are averages of 10 repetitions±sd. ■ plants in the course of repair from radiation injuries, □ control plants. B = budding; BF = beginning of flowering [≤ 25% of plants had flowers]; BPF = beginning of pod formation [≤ 25 % of plants began to develop pods]

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GROWTH AND PHYSIOLOGICAL RESPONSES OF WILD OATS TO THE ALLELOPATHIC POTENTIAL OF WHEAT

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Greenhouse experiments were conducted to discover the effects of the tissue extracts and competition of wheat (*Triticum aestivum* L.) on the growth attributes of wild oats (*Avena fatua* L.). Water-soluble toxins from living tissues of wheat significantly decreased the growth attributes of wild oats. In comparison with the control, the irrigation of wild oat plants with shoot extracts resulted in a significant decrease in their total biomass, pigment, carbohydrate and protein contents, but the inhibitory effects of the root extract were non-significant. Along with allelopathy, competition has a marked effect on the growth reduction of wild oat plants.

Key words: allelopathy, competition, *Avena fatua*, carbohydrates, allelochemicals

Introduction

During the past four decades, the increasing use of herbicides in agriculture has led to the evolution of weed biotypes less sensitive to the chemical effects of herbicides, which also lead to environmental pollution and health hazards (Chou et al., 1977; Narwal, 1992, 1996; Hegazy, 1994). To overcome these problems, agronomists have started searching for alternatives to herbicides. One such alternative is the use of crop allelopathic strategies, through the development of crop varieties having a greater ability to smother weeds, and the use of natural phytotoxins from plants as herbicides (Chou, 1989; Dilday et al., 1996).

Many nations need to convert currently unsustainable agriculture systems into sustainable ones. Sustainable development strategies for the agroecosystem need to manage and limit pest damage by applying biological principles of sustainability. These strategies make use of the allelopathic interaction between weeds and crops.

Wheat straw is closely associated with allelopathy (Harper and Lynch, 1981; Liebel and Worsham, 1983; Shilling et al., 1984). Sixty-six compounds have been identified from the straw of wheat (Gaspar and Neves, 1995). These compounds are carboxylic acid methyl esters, phenolic acids and triterpenoids. Wheat–weeds interactions have been the subject of many studies (Gonzalez-Ponce and Lamela, 1987; Medd, 1990; Satorce and Snaydon, 1991; Cudney et al., 1991; Dunan and Zimdahl, 1991; Gonzalez-Ponce et al., 1992; Kirkland, 1993; Qasem, 1993; Grace, 1995; Ben-Hamouda et al., 1995a, b; Gaspar and Neves, 1995). Whether these studies considered allelopathy or competition in the wheat–wild oat interaction, they were concerned mainly with the effect of increasing infestations of wild oat species on the wheat crop and ignored the

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effect of the latter on weed growth, which could occur due to the compounds liberated by the crop. Since allelopathic studies of wheat on its associated weeds are still limited, the present study was undertaken to test the potential allelopathic effect of wheat on wild oats, which ranks as one of its most serious weeds.

Materials and methods

Wild oat (*Avena fatua* L.) seeds were sown in pots 25 cm in diameter and 28 cm in depth. They were filled with soil collected from the natural field shortly before the start of the experiments. The soil was excavated from between a depth of 5–20 cm and had a pH of 7.8, an organic matter content of 1.7% and an electric conductance of 356 µmhos/cm, while the mechanical analysis showed 66% and, 5% silt and 29% clay. Two separate experiments were designed and the pots were placed side by side in a growth chamber with a temperature of 22±2°C and 170 µmol m^{-2} s^{-1}photon flux density during an 11 h photoperiod.

Experiment 1

Randomly selected wheat plants (*Triticum aestivum* L.) grown by wheat farmers around the University of Sohag Campus in Egypt were collected intact at the vegetative phase prior to flowering. They were washed gently with distilled water, blotted, separated into shoots and roots, and chopped into pieces about 1 cm long. An aqueous plant extract (30%) was prepared by mixing 30 g of the plant parts (root and shoot) with 100 ml of distilled water and agitating it on a rotating shaker. The extracts were filtered (Whatman No. 2 filter paper), centrifuged at 12,500 rpm for 20 min at 8°C and then sterilized by passing through a 0.45 µ Millipore filter (Wardel et al., 1993). The growing seedlings of wild oats were watered with the wheat extract in a randomized block design with three replicate pots for each treatment. The plants were grown for 30 days, then harvested and oven dried at 80°C. Successive weighing was carried out until each sample reached constant dry mass, which was expressed as g $plant^{-1}$. Before the plant harvest, the following growth criteria were measured: plant height expressed in cm, leaf areas determined and expressed as cm^2 $plant^{-1}$ (Watson and Watson, 1953), photosynthetic pigments (chlorophyll a, b and carotenoids) determined by the spectrophotometric method (Metzner et al., 1965) and carbohydrate and protein contents determined colorimetrically according to Badour (1959) and Lowry et al. (1951), respectively.

Experiment 2

Seeds of wheat were sown directly in the middle of the pots. To ensure that the emergence of the wheat seedlings coincided with that of wild oats, seeds of wild oats were germinated in separate pots and five seedlings were introduced into the pots, where they were planted evenly around the wheat plants. Five densities of wheat (2, 4, 6, 8 and 10 plants/pot) were used. The densities tested were 5A+2T, 5A+4T, 5A+6T, 5A+8T and 5A+10T where A = *Avena* and T = *Triticum*. The wild oat plants were harvested 30 days after planting. Growth measurements were made as outlined in the first experiment.

All data were subjected to standard analysis of variance using the "Statgraphics" statistical analysis software (STATG, 1991). Comparisons of the main effects were performed using the least significance difference method. Significance levels of $P<0.05$ and 0.01 were used for all the statistical procedures.

Results and discussion

Experiment 1

The plant height, leaf area and total mass of the wild oat plants were reduced when irrigated with aqueous extracts of either shoot or root tissue of wheat, as compared to the control. The reduction was more significant in the case of shoot extract than root (Fig. 1a, b, c). This reduction suggested that during the extraction toxic substances were released from the wheat tissues and interfered with the cell division and elongation of wild oats. Therefore, reduced root cell growth may lead to a decrease in mineral nutrient uptake, nutrient absorption, active ion transport mechanisms, and the transport of nutrients from the root to other plant parts, finally reducing the growth and development of wild oats. This reduction in the dry mass of the plants could explain the differences observed in the growth of wild oats.

Control plants of wild oats had higher pigment contents than treated plants (Fig. 1d). Chlorophyll a and chlorophyll b were both strongly inhibited by wheat shoot extract, while a slight decrease in carotenoids was also recorded. A comparable effect was shown for the root extract, but this was non-significant. In this respect Colton and Einhellig (1980) and Patterson (1981) reported that allelochemicals caused a marked reduction in the chlorophyll contents of tested plants through their effects on the biosynthesis and denaturation of chlorophyll molecules.

The results demonstrated that both soluble and insoluble carbohydrates responded differently to different tissue extracts. While the shoot extract significantly reduced the soluble carbohydrate content of wild oats, the root extract had a non-significant effect (Fig. 1e). Wild oat plants were susceptible to wheat allelochemicals and exhibited a significant reduction in the total carbohydrate content. This reduction may be due to the direct or indirect effects of allelochemicals on net photosynthesis. These effects may operate through the inhibition of stomatal opening and CO_2 uptake (Zelitch, 1967; Einhellig, 1971; Lodhi and Nickell, 1973), and/or through the inhibition of coupled electron transport and both cyclic and non-cyclic photophosphorylation (Arntzen et al., 1974).

Wild oat plants exhibited a reduction in their total protein contents when treated with wheat tissue extracts. It can be seen that both soluble and insoluble protein contents were significantly reduced after the application of different wheat extracts (Fig. 1e). It was found that many allelopathic compounds could reduce growth by inhibiting the incorporation of many amino acids into protein (Van Sumere et al., 1971).

As revealed by the results, all the growth attributes of wild oats were significantly inhibited by the toxic effects of the wheat shoot extract, and non-significantly inhibited by those of the root extract. Accordingly, it is suggested that the toxic substances of wheat are mainly synthesized in the shoot, where they are concentrated, while small amounts of these substances may move to the root, where they exert a non-significant effect on the growth attributes of wild oats.

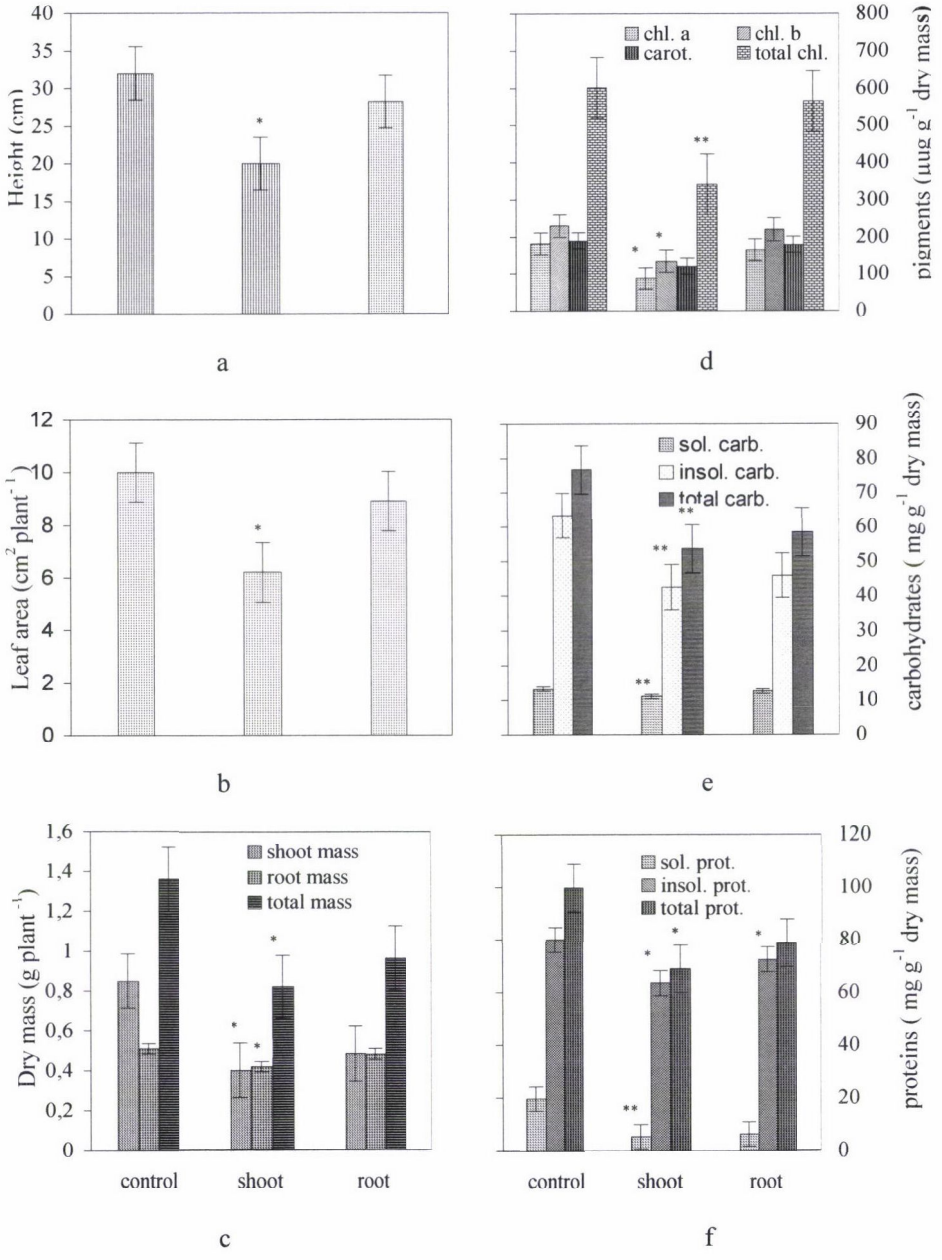

Fig. 1. Effect of shoot and root extract of wheat on the mean ±SD of the growth attributes of wild oats. *, ** significant at $P<0.05$, and $P<0.01$, respectively

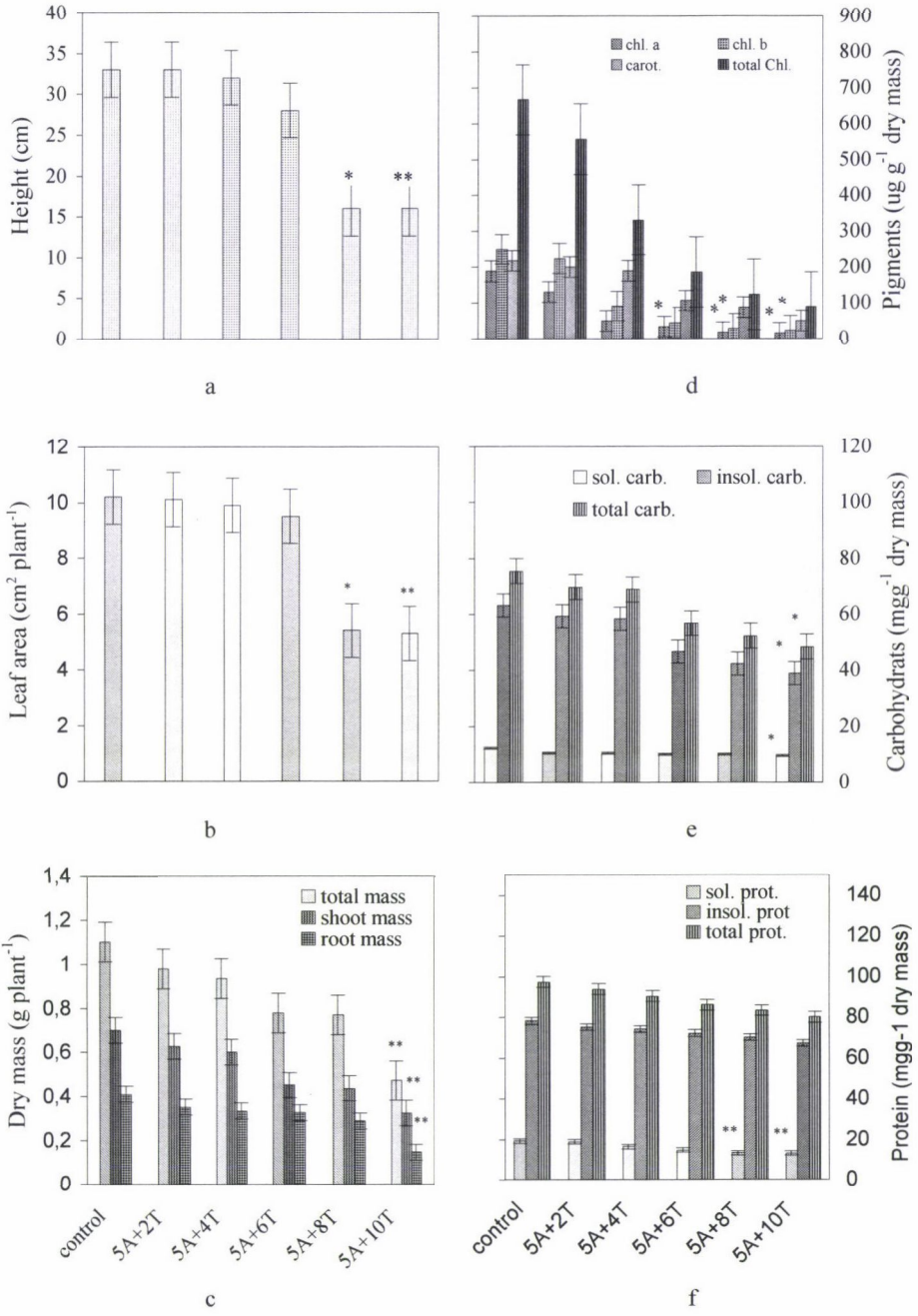

Fig. 2. Effect of wheat density (T) on mean ±SD of the growth attributes of wild oat (A).
*, ** = significant at P<0.05 and P<0.01, respectively

Experiment 2

Here, the inhibition effects in the growth attributes of wild oats are clearly differ from those in the first experiment. As the number of wheat plants increased, there was a trend towards a decrease in the growth attributes of wild oat plants. Low wheat density had a non-significant effect on the weed growth, while a highly significant reduction in the plant height, leaf area and total mass of wild oats was recorded in replicates with a density of 5A+10T (Fig. 2a, b, c). As compared with the control, an increase in wheat density caused a marked inhibition in the biosynthesis of chlorophyll, carbohydrates and protein in wild oats (Fig. 2d, e, f). These results show that plant density is an important factor in determining the competitive ability of cereals. This interference capacity affected the height, leaf area, dry mass, pigment, carbohydrate and protein contents of wild oat plants more than allelopathic potential alone. Therefore, the results support the findings of many authors (Muller, 1969; El-Khatib, 1998; Hegazy, 1997) who reported that both allelopathy and competition are involved in species interactions. They are also in agreement with the results of Radford et al. (1980) and Wilson and Wright (1990) who reported that an increase in the seeding rate of wheat could be used for the low-cost control of wild oats with fewer crop yield losses.

In conclusion, it can be said that the growth attributes of wild oats are aggressively reduced by wheat interference. The present study therefore indicates that allelopathy is not operative alone, but that allelopathy offers the most reasonable explanation of the growth response observed. In addition, the present study provides the outline of a new strategy for the agro-ecosystem, taking into consideration the allelopathic potential of wheat in controlling wild oat infestations and/or in facilitating the selection and development of tolerant or resistant wheat varieties. According to Altier and Doll (1978) this method is better adjusted to energy, economic and ecological requirements in the actual context of agro-ecosystem management.

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EFFECT OF IRON–MANGANESE INTERACTION ON THE YIELD AND CONTENT OF Fe AND Mn IN MAIZE (*ZEA MAYS* L.)

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A greenhouse study was conducted to evaluate the influence of Fe and Mn application on the dry matter yield, and on the content and uptake of these elements in maize grown on Fe- and Mn-deficient alkaline soils. There were five levels of Fe (0, 25, 50, 100 and 200 mg Fe kg^{-1} soil as $FeSO_4.7H_2O$) and four levels of Mn (0, 50, 100 and 200 mg Mn kg^{-1} soil as $MnSO_4.H_2O$). The highest yield was obtained with the combined application of 50 mg Fe and 100 mg Mn kg^{-1} soil, while the further application of either of these micronutrients decreased the yield significantly. Higher rates of Fe decreased the content and uptake of Mn in the plants and resulted in a significant decrease in yield. Similarly, higher rates of applied Mn resulted in a significant decrease in the content and uptake of Fe in the plants. The significant reduction in the dry matter yield and in Mn uptake indicated the antagonistic effect of higher doses of iron fertilization on Mn utilization by maize.

Key words: Fe–Mn interaction, alkaline soil, maize, dry matter yield

Introduction

An antagonistic relationship between Fe and Mn has been reported in soils and plants (Gupta, 1972; Heenan and Campbell, 1983) and they have been found to influence the yields of crops in various ways. Baser and Saxena (1970) reported an increase in the rice yield after the application of Fe and Mn. Sometimes the ratio of Fe and Mn, either in the soil or in plants, had no significant impact on the crop yields; only when they were added in optimum concentrations was a significant difference observed. A high level of Mn reduced the tissue Fe concentrations and induced iron deficiency symptoms in pineapple (Sideris and Young, 1949), but not in tomatoes (Sanchez-Raya et al., 1974). These contrasting observations may be due to the redox reactions in the soil or to the lack of suitable chelates to maintain iron availability. In addition, the mobility of both Fe and Mn within the plant is reduced, but can be influenced by the levels of macro- and micronutrients. However, practically no information is available on this aspect in relation to maize. The aim of this study was to determine the effect of iron and manganese interaction on the yield and uptake of these ions in maize grown in a coarsely textured soil.

Materials and methods

A pot culture experiment was conducted using a Fatehpur loamy sand containing 72.9% sand (>50 μm), 18.2% silt (2–50 μm) and 8.7% clay (< 2μm). The soil belongs to the great group Ustipsamments. The soil had a pH($H_2$0) of 8.3 and an electrical conductivity of 0.2 dS m^{-1} in a

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1:2 soil:water suspension at 25°C. The contents of organic carbon and calcium carbonate were 0.25 and 0.60% respectively. Soil available phosphorus was 8 mg kg^{-1} soil. Available iron and manganese extracted by the DTPA method of Lindsay and Norvell (1978) were 4.8 and 2.4 mg kg^{-1}, respectively.

Polyethylene-lined earthen pots were filled with 4 kg soil. The treatments comprised five levels of Fe (0, 25, 50, 100 and 200 mg Fe kg^{-1} soil from $FeSO_4.7H_2O$) and four levels of Mn (0, 50, 100 and 200 mg Mn kg^{-1} soil from $MnSO_4.H_2O$). These levels of Fe and Mn were tested in all possible combinations. All the pots received a basal application of 120 mg N, 26 mg P and 25 mg K kg^{-1} soil in the form of urea, potassium dihydrogen orthophosphate and potassium chloride, respectively. There were three pots for each treatment and the total number of pots in the experiment was 60. The required quantity of salts was applied in solution form to 4 kg soil and thoroughly mixed before filling the pots. Maize (cv. Partap) was grown as a test crop. Eight seeds were sown in each pot and were thinned to three after emergence. The soil was initially adjusted to approximately 60% of the saturation moisture content and the pots were subsequently watered as and when required. The crop was harvested by cutting at the soil surface 55 days after germination. Representative soil samples were taken from each pot after harvesting the crop.

The plant samples were washed successively with 0.1 N HCl, distilled and deionized water and dried at 70°C. The oven-dried weight was recorded. The samples were ground in a Wiley Mill fitted with stainless steel blades to pass a 20 mesh stainless steel sieve. The plant samples were wet-ashed with a nitric-perchloric-sulphuric acid mixture. The soil samples were analysed for available Fe and Mn by extraction with DTPA solution (0.005 M diethylene triamine pentaacetic acid + 0.1 M triethanol amine + 0.01 M $CaCl_2$ adjusted to pH 7.3 with HCl) using a soil to solution ratio of 1:2 and a shaking time of 2 hours (Lindsay and Norvell, 1978). The contents of organic carbon, calcium carbonate and available phosphorus in the soil samples were determined using the procedures described by Black (1965).The contents of Fe and Mn in the plant and soil extracts were measured by atomic absorption spectrophotometry.

Results and discussion

Dry matter yield

The dry matter yield was found to be significantly affected by the interaction between Fe and Mn. In the absence of added Fe, the application of Mn up to 100 mg kg^{-1} soil significantly increased the dry matter yield of maize, which, however, decreased after the application of 200 mg Mn kg^{-1} soil (Table 1). Similarly, in the absence of added Mn, when Fe was applied up to 50 mg kg^{-1} soil, the yield significantly increased over the control treatment but decreased non-significantly after the application of 100 or 200 mg Fe kg^{-1} soil. This indicated that the soil was more deficient in available Mn than in Fe. The maximum dry matter yield was obtained with the combined application of 50 mg Fe and 100 mg Mn kg^{-1} soil. The application of either of these nutrients beyond these levels had a negative effect on biomass production, as reflected by the marked decline in dry matter yield. This yield depression most pronounced at the highest level of application. There was a 30.9% reduction in yield compared with the 50 mg Fe and 100 mg Mn kg^{-1} soil level after the application of 200 mg Mn kg^{-1} soil, while the application of 100 or 200 mg Fe kg^{-1} soil decreased the yield by 21.8 and 29.1%, respectively, compared with the optimum treatment (Table 1).

Table 1
Effect of Fe and Mn application on the dry matter yield, Fe content and Fe uptake in plants

Rates of Fe application (mg kg^{-1} soil)	Rates of Mn application (mg kg^{-1} soil)				
	0	50	100	200	Mean
	Dry matter yield (g pot^{-1})				
0	3.4	4.2	5.0	3.6	4.1
25	4.3	4.7	5.1	4.1	4.6
50	4.5	4.9	5.5	3.8	4.7
100	4.0	4.6	4.3	3.3	4.1
200	3.2	4.5	3.9	2.6	3.6
Mean	3.9	4.6	4.8	3.5	
	Fe content in plant (μg g^{-1})				
0	200	237	291	262	247
25	270	304	325	300	300
50	295	309	316	288	302
100	310	320	340	298	317
200	350	308	294	242	317
Mean	285	296	313	278	
	Fe uptake in plant (mg pot^{-1})				
0	0.68	0.99	1.45	0.94	1.01
25	1.16	1.43	1.66	1.23	1.37
50	1.33	1.51	1.74	1.09	1.42
100	1.24	1.47	1.46	0.98	1.29
200	1.12	1.39	1.15	0.63	1.07
Mean	1.11	1.36	1.49	0.97	

LSD at P=0.05	Dry matter yield	Fe conc.	Fe uptake
Fe	0.4	50	0.26
Mn	0.3	non significant	0.23
Fe × Mn	0.8	non significant	0.52

The mean dry matter yield increased significantly with the application of 50 and 100 mg Mn kg^{-1} soil. However, the difference between these two levels was non-significant. At an application level of 200 mg Mn kg^{-1} soil, the yield decreased sharply. The reduction in yield at 200 mg Mn kg^{-1} soil, compared to the 100 mg Mn kg^{-1} soil application rate, was 27.1%. Similarly, an average response to Fe was observed up to 50 mg Fe kg^{-1} soil, while at 200 mg Fe kg^{-1} soil there was a significant decrease in yield. According to Lingle et al. (1963) the concentration of one element in the nutrient solution or in the plant tissues influences the absorption and translocation of other elements and hence the growth and the yield response. The adverse effect of Fe on the yield may be due to the antagonistic effect of Fe on the availability and uptake of Mn by maize plants. Gupta (1972) reported that there was a 50% reduction in barley yield after the application of 400 mg Fe kg^{-1} soil. Singh and Yadav (1980) also reported that a higher rate of Fe application decreased the yield of sorghum significantly.

Fe content and its uptake

There was a significant increase in the plant Fe content with an increase in the rates of Fe application at all levels of applied Mn, while increasing rates of Mn application increased the Fe content in the plants non-significantly. The mean Fe contents were 247, 300, 302, 317 and 298 μg g^{-1} at 0, 25, 50, 100 and 200 mg Fe kg^{-1} soil, respectively (Table 1). A significant increase in the Fe content in the plants was observed at 25 mg Fe kg^{-1} soil, while the further addition of Fe did not affect the Fe content in the plants significantly. The highest plant Fe content of 340 μg g^{-1} was observed after the combined application of 100 mg each of Fe and Mn kg^{-1} soil, while the further application of either of the elements decreased the Fe content in the plants. Manganese application had a non-significant influence on the Fe content in the plants, which may be due to the dilution effect. An increase in Fe concentration with Fe application has been reported by several workers (Singh and Yadav, 1980; Heenan and Campbell, 1983).

The mean Fe uptake was found to be significantly increased over the control treatment by the application of 50 mg Fe or 100 mg Mn kg^{-1} soil, after which it decreased. The increase in Fe uptake over the control treatment was of the order of 0.36, 0.41, 0.28 and 0.06 mg pot^{-1} at 25, 50, 100 and 200 mg Fe kg^{-1} soil, respectively. The increase in Fe uptake with the increase in the level of Fe may be attributed to the higher availability of Fe in the growth medium as well as to a decrease in soil pH. The Fe uptake in the plants was significantly decreased at the highest Mn level (200 mg Mn kg^{-1} soil) as compared to its uptake at 100 mg Mn kg^{-1} soil. The decrease in mean Fe uptake over the control treatment was 12.6% after the application of 200 mg Mn kg^{-1} soil. A decrease in Fe uptake at higher rates of Mn application was also reported in sorghum (Kuo and Mikkelson, 1981) and soybean (Heenan and Campbell, 1983). These results were confirmed by the work of Romheld and Marschner (1986), who reported that the rate of Fe uptake by iron-deficient cucumber plants rapidly declined with an increase in pH, as the result of the inhibitory action of high pH on Fe^{+} reductase at the root surface. Although iron is present in abundance in most well-aerated soils, it is not available for plant uptake, primarily because it is mostly insoluble. To make iron more available, plants have evolved various adaptation mechanisms that mobilize iron at the root-soil interface, i.e. the rhizosphere. By conducting experiments with young barley plants grown in water culture, Clarkson and Sanderson (1978) showed that the removal of an external supply of iron for 17 days resulted in a marked increase in the capacity of the roots to absorb Fe^{3+} and to translocate it to the shoots. In the present work, the low value of Fe concentration and Fe uptake in maize plants in the no Fe treatment could be the result of the high initial pH of the soil as compared to the decrease in soil pH induced by the addition of ferrous sulphate.

Mn content and its uptake

The manganese content in the plants increased significantly with the increase in Mn application. The mean Mn content was 26, 50, 60 and 69 μg g^{-1} at 0, 50, 100 and 200 mg Mn kg^{-1} soil, respectively. There was a successive

increase in Mn content with the increasing rates of applied Mn. In the absence of Fe application, the plant Mn content also increased from 32 μg g^{-1} in the control to 69 μg g^{-1} at the highest level of applied Mn (Table 2). In the absence of Mn application, the Mn content in the plant decreased successively with increasing rates of applied Fe. The mean Mn uptake initially increased with Fe application at 25 and 50 mg Fe kg^{-1} soil, after which it decreased. At 200 mg Fe kg^{-1} soil, there was a marked, significant decrease in the Mn content in the plants over the control treatment. The increase in Mn content in the plants with the increase in the level of Mn application was expected because of its increased availability to the plants. The decrease in Mn content at higher rates of Fe application may be due to the competition of these two ions at the carrier sites, as both are involved in oxidation-reduction reactions. A decrease in Mn content with Fe application was also reported in oats (Singh and Dahiya, 1980) and soybean (Moraghan, 1985).

Table 2
Effect of Fe and Mn application on Mn content and Mn uptake in plants

Rates of Fe application (mg kg^{-1} soil)	Rates of Mn application (mg kg^{-1} soil)				
	0	50	100	200	Mean
	Mn content in plant (μg g^{-1})				
0	32	55	58	69	53
25	30	59	61	75	56
50	27	52	65	77	55
100	24	45	63	69	50
200	18	40	51	55	41
Mean	26	50	60	69	
	Mn uptake in plant (mg pot^{-1})				
0	0.11	0.23	0.29	0.25	0.22
25	0.13	0.28	0.31	0.31	0.26
50	0.12	0.25	0.36	0.29	0.25
100	0.09	0.21	0.27	0.23	0.20
200	0.06	0.18	0.20	0.14	0.14
Mean	0.10	0.22	0.29	0.24	

LSD at P=0.05	Mn content	Mn uptake
Fe	6	0.05
Mn	5	0.04
Fe × Mn	12	0.10

The average Mn uptake in the plants significantly increased with the rate of Mn application except at the highest level (200 mg Mn kg^{-1} soil), when it decreased significantly compared with the 100 mg Mn kg^{-1} soil treatment (Table 2). The increase in Mn uptake was of the order of 0.13 and 0.19 mg pot^{-1} at 50 and 100 mg Mn kg^{-1}, respectively, as compared to the no Mn treatment. Fe application increased the mean Mn uptake by 18.0 and 13.6% respectively, at 25 and 50 mg Fe kg^{-1} soil, after which there was a decrease of 9.0 and 36.4% in Fe uptake with the application of 100 and 200 mg Fe kg^{-1} soil, respectively,

compared to the control treatment. The interaction effect between Fe and Mn was also significant. The maximum Mn uptake (0.36 mg Mn pot^{-1}) was observed with the combined application of 50 mg Fe kg^{-1} soil and 100 mg Mn kg^{-1} soil (Table 2). Increasing the concentration of either Mn or Fe decreased the Mn uptake by the plants. As the DTPA-available Fe in the soil increased from 5.2 mg kg^{-1} in the control treatment to 12.8 mg kg^{-1} at the highest rates of Fe application, the plant Mn content decreased from 32 μg g^{-1} in the control treatment to 18 μg g^{-1} with the application of 200 mg Fe kg^{-1} soil. The adverse effect of Fe on the Mn uptake was also reported in barley (Vlamis and Williams, 1964) and sorghum (Singh and Yadav, 1980). The decrease in Mn uptake at 200 mg Fe kg^{-1} soil application resulted by and large from the combined influence of lower dry matter yield and the less intensive absorption of Mn because of ionic competition.

Soil pH and available Fe and Mn

The ferrous sulphate application decreased the soil pH from 8.3 to 7.9, whereas manganese sulphate application had a negligible effect on soil pH (Table 3). The decrease in soil pH with $FeSO_4.7H_2O$ addition may be due to the reclamation effect of this fertilizer (Milap Chand et al., 1977). After the crop harvest, it was found that the native soil Fe tended to increase with cropping from 4.8 to 5.2 mg kg^{-1} soil, whereas soil Mn exhibited no such effect. The increase in extractable Fe could be attributed to the activity of micro-organisms and plant roots producing chelating agents which tend to hold more Fe in solution (Follett and Lindsay, 1971). At each level of Mn application, although an increasing rate of applied Fe markedly and successively increased the DTPA-available Fe, a significant increase occurred at 50 mg Fe kg^{-1} and above. At each level of Fe application, increasing rates of Mn caused a slight, non-significant increase in DTPA-extractable Fe. Increasing rates of Mn significantly and successively increased the DTPA-available Mn at each level of Fe application. However, increasing rates of applied Fe resulted in a significant decrease in available Mn at 200 mg Mn kg^{-1} soil compared with the control treatment.

Table 3
Soil pH and recovery of added iron and manganese by DTPA extractant after the crop harvest

Treatment (mg kg^{-1} soil)	Soil pH (H_2O)	DTPA-extractable Fe or Mn (mg kg^{-1} soil)	% Recovery
		Iron	
0	8.3	5.2	–
25	8.2	5.6	1.6
50	8.2	7.6	4.0
100	8.1	9.4	4.2
200	7.9	12.8	3.8
		Manganese	
0	8.3	2.5	–
50	8.3	7.6	10.2
100	8.2	11.8	9.3
200	8.2	16.2	6.8

Fe and Mn fertilization marginally increased their respective available contents in the soil. This indicates that the residual value of these fertilizers in alkaline soils is very low. Follett and Lindsay (1971) reported that most of the soil-applied inorganic iron fertilizers, such as $FeSO_4.7H_2O$, are rapidly fixed in the soil and become unavailable. The recovery of added Fe was only 20% in neutral soils after one week of application. Manganese was fixed to a lesser extent than iron. This is due to the fact that Mn fertilizers are rapidly oxidized and precipitated as insoluble manganese oxide under neutral and alkaline conditions. The present study suggested that higher rates of Fe application had an antagonistic effect on Mn availability to maize, whereas Mn had a non-significant influence on Fe availability to plants.

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PRODUCTION AND CYTOGENETIC ANALYSIS OF *TRITICUM AESTIVUM* L. × *TRITICUM TIMOPHEEVII* ZHUK. HYBRIDS, AMPHIPLOIDS AND BACKCROSS PROGENIES

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Triticum timopheevii and *Triticum araraticum* × wheat hybrids were produced with three cultivated wheat varieties, Mv 9, Mv 15 and Amor, and one wheat line, Mv 9 kr1, to develop alien addition and translocation lines with the aim of transferring disease resistance into cultivated wheat. The reciprocal crosses were carried out in all combinations.

The morphology of the F_1 spikes in most combinations was an intermediate hybrid form, while in some combinations other forms such as square head, awned or awnless were observed. The hybrids showed good vegetative growth and good tillering ability. The number of mitotic chromosomes in all F_1 hybrids was 35, and the number of meiotic chromosomes, taking into account the number of fragments, was 35 as well. The chromosome pairing of the F_1 hybrids fitted the expected values. The mitotic chromosome number of the amphiploid was 70 and chromosome pairing was $5.88^{I} + 8.42^{II}(rod) + 21.14^{II}(ring) + 1.5^{III} + 0.02^{Telo} + 0.05^{Fragment}$. The chiasma frequency was 53.70. Backcross progenies with 43 and 44 chromosomes were produced, but he meiotic pairing in these lines did not confirm the assumption that these were monosomic and disomic alien addition lines. Further studies are in progress to identify which chromosomes of *T. timopheevii* are present in these lines.

Key words: interspecific hybridization, *Triticum aestivum*, *Triticum timopheevii*, meiosis

Introduction

The gene pool of cultivated bread wheat has been greatly eroded by modern cultivation techniques. Many wild wheats can be crossed successfully with bread wheat and represent a large reservoir of useful traits that can be exploited for wheat improvement (Friebe et al., 1996).

Triticum timopheevii Zhuk. is considered to be a valuable source of tolerance to biotic and abiotic stresses. A high level of drought tolerance was reported in *T. timopheevii* (Grignac, 1965). Tetraploid wheat species, particularly *T. timopheevii* Zhuk. and the wild tetraploid *T. araraticum* Jakubz., have also contributed to wheat improvement in the past and will probably continue to be sources of useful genes in the future (Jauhar, 1993). *Triticum timopheevii* Zhuk. var. *timopheevii* is a source of stem rust resistance (McIntosh and Gyárfás, 1971).

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The first wheat - *T. timopheevii* amphiploid was produced by colchicine treatment and designated as *Triticum borisovi* Zsebr. by Zsebrak (1944). Later, Belea (1961) and Tavrin (1961) also produced wheat-*T. timopheevii* amphiploids. Allard and Shands (1954) reported on the inheritance of stem rust in two hexaploid lines, CI12632 and CI12633, with resistance from *T. timopheevii*. The wheat cultivar Timvera, with this type of resistance, was developed in Australia (Watson and Luig, 1958).

The cultivar Arthur and some related soft red winter wheats that were widely grown in the eastern USA had resistance from CI12633 (Patterson et al., 1975). Attempts were made to determine the genetic variability for rust resistance in accessions of *T. timopheevii* and *T. araraticum*. Morris and Sears (1967) considered *T. araraticum* to be a wild form of *T. timopheevii*. McIntosh and Gyárfás (1971) tested a group of *T. timopheevii* and *T. araraticum* accessions with Australian and North American isolates of *P. graminis*. Spontaneous single, double and triple chromosome substitution lines of *T. araraticum* chromosomes 3At, 2G, 4G, 5G and 6G were isolated in *T. aestivum* cv. Wichita by Gill et al. (1988). All the substitution lines were fully fertile and morphologically normal. The most frequently substituted chromosome was 6G (Badaeva and Gill, 1995).

The aim of the current experiments was to produce new hybrids with different accessions of *T. timopheevii and T. araraticum* to transfer disease resistance into cultivated bread wheat varieties.

Materials and methods

Plant material

Experiments were carried out in the field and in phytotron chambers in Martonvásár. Three cultivated wheat (*T. aestivum* L.) (2n=6x=42) varieties: (Martonvásári 9 = Mv 9, Martonvásári 15 = Mv 15, Ámor), one wheat line (Mv 9 kr1) possessing the crossability gene *kr1* (Molnár-Láng and Sutka, 1996), 11 accessions of *T. timopheevii* Zhuk. (TG 1–TG 11) and 10 accessions of *T. araraticum* Jakubz. (TG 12–TG 21) were used. These accessions are maintained in the Cereal Gene Bank of the Agricultural Research Institute of the Hungarian Academy of Sciences.

Pollination, embryo culture

The seeds were sown in autumn in the field. The spikes were emasculated and half of the spike was cut off. The detached spikes were taken into the phytotron chamber (20°C, 80% humidity) and were put into Hoagland nutrient solution. Florets were pollinated with the "twirling" method by the donor parents three to five days after emasculation both in the field and in the phytotron. From all combinations a number of immature embryos were excised and placed on B5 medium, while the others remained in the head until harvesting. The embryos were placed in a dark incubator at 26°C.

Colchicine treatment

F_1 plantlets were put into colchicine solution with a concentration of 0.04% overnight (12 hours) at a temperature of 15°C.

Cytological analysis

The Feulgen staining method was used to analyse the chromosome number in mitosis and the chromosome associations in meiosis of the F_1 hybrids, the amphiploids and the BC_1 and BC_2 progenies.

Results

1. Morphological description and cytogenetic analysis of the T. aestivum x T. timopheevii hybrids

The F_1 hybrids showed great diversity between parental values. The plant height was intermediate between that of the parents and the hybrids generally showed good tillering ability. The morphology of the spikes in most combinations was an intermediate form, while in some combinations unusual forms, such as square head, awned, awnless or red stems, were observed. Some of the hybrids were resistant to powdery mildew.

The chromosome number and chromosome associations at metaphase I in meiosis of the pollen mother cells in different F_1 hybrids are presented in Table 1.

The number of mitotic chromosomes in all the F_1 hybrids was 35. In all combinations the number of meiotic chromosomes, taking into account the number of fragments, was also 35, which is equal to the expected chromosome number in the F_1 generation., The grand mean of the chromosome number of all F_1 combinations in both directions was calculated. Both *T. timopheevii* and *T. araraticum* were designated as *T. timopheevii* and other wheat cultivars as *T. aestivum*. The F_1 hybrids in both directions had the expected mitotic chromosome number, 2n=5x=35. The meiotic pairing in the pollen mother cells of F_1 hybrids in *T. timopheevii* × *T. aestivum* combinations was $15.78^{I} + 5.95^{II}$ (rod) + 3.03^{II} (ring) + 0.31^{III} + 0.05^{IV} + 0.18 fragments. The frequency of chiasmas per cell was 12.87. The reciprocal hybrid had the following chromosome pairing: $17.22^{I} + 4.95^{II}$ (rod) + 3.43^{II} (ring) + 0.29^{III} + 0.03^{IV} + 0.02 telo. The number of chiasmas was 12.06. In general there was no significant difference between the reciprocal crosses in chromosome pairing data.

2. Chromosome number of amphiploids and further generations

The mitotic chromosome number and chromosome configurations in the meiosis of the amphiploids, BC_1, BC_2 progenies and addition lines are illustrated in Table 2. The mitotic chromosome number of the amphiploids (Mv 9 kr1 × *T. araraticum*) was 70. The chromosome pairing was $5.88^{I} + 8.42^{II}$ (rod) + 21.14^{II} (ring) + 1.5^{III} + 0.02 telo + 0.05 fragment. The chiasma frequency was 53.70. The number of chromosomes in the root tips of the first backcross was 56, and the meiotic chromosome association was 9.44 I + 10.6 II rod + 10.6 II ring + 0.33 III + 0.7 IV + 0.1 fragment with a chiasma frequency of 34.57 per cell. The number of chromosomes in BC_2 ranged from 38–48. Plants with 43 chromosomes in BC_2 were selected to develop monosomic alien addition lines. These were then selfed and finally plants with 44 chromosomes were isolated which might be alien disomic addition lines.

Table 1

Mean chromosome pairing at metaphase I in the meiosis of the F_1 hybrids *Triticum aestivum* × *Triticum timopheevii*

Combinations	No. of cells	Mitotic chromos. no.	Chromosome associations						Telo-centric	Fragments	Chiasma	Total
			Uni-valents	Bivalents			Tri-valents	Quadri-valents				
				Ring	Rod	Total						
T .timopheevii × Mv 9	752	35	15.02	3.60	5.54	9.14	0.38	0.10	–	0.19	13.8	34.84
T. araraticum × Mv 9	48	35	15.76	3.71	5.19	8.90	0.33	0.04	–	0.09	13.39	34.71
T. araraticum × Mv 15	34	35	16.76	1.40	7.24	8.64	0.12	–	–		10.28	34.52
T.araraticum × Amor	153	35	16.52	2.71	5.29	8.00	0.57	0.14	–	0.38	12.84	34.79
T.araraticum × Mv 9 kr1	125	35	17.28	3.45	4.95	8.40	0.41	0.01	–	0.43	12.74	34.41
T .timpheevii × Mv 15	6	35	13.33	3.33	7.50	10.83	–	–	–		14.16	34.99
Mv 9 kr1 × *T. timopheevii*	71	35	16.30	3.52	5.50	9.02	0.23	–	–		13	35.03
Mv 9 kr1 × *T. araraticum*	445	35	18.15	2.77	4.40	7.17	0.50	0.06	0.02		11.12	34.23
T. timpheevii × *T. aestivum**	925	35	16.02	3.58	5.22	8.81	0.37	0.05	–	0.24	13.31	34.90
T. aestivum × *T. timpheevii**	516	35	17.22	3.43	4.95	8.38	0.29	0.03	0.02		12.06	34.97

*Grand mean of chromosome number over similar combinations

Table 2

Mean chromosome pairing of amphiploid, BC_1, BC_2 and alien addition lines at metaphase I of meiosis

Combinations	No. of cells	Mitotic chromos. no.	Chromosome associations						Telo-centric	Fragments	Chiasma	Total
			Uni-valents	Bivalents			Tri-valents	Quadri-valents				
				Ring	Rod	Total						
Mv 9 kr1 × *T. araraticum*$^+$	284	70	5.88	21.14	8.42	29.56	1.5		0.02	0.05	53.7	69.5
[(Mv 9 kr1 × *T .araraticum*) × Mv 9 kr1] BC_1	45	56.4	9.44	10.6	10.6	21.21	0.33	0.7		0.1	34.57	55.65
[(Mv 9 kr1 × *T.araraticum*) × Mv 9 kr1] BC_2	50	38-48										
T .timopheevii × Mv 9*	45	43	2	15.6	3.4	19	1	0.05				43.2
T. timopheevii × Mv 9**	40	44	3.60	16.7	2.83	19.53	0.30	0.15				44.16
T. araraticum × Mv 9 kr1	30	44	2	11	10	21						44

$^+$amphiploid; *,**: progenies with 43 and 44 chromosomes, respectively

Discussion

The chromosome numbers and configurations were analysed at metaphase I of mitosis and meiosis of the F_1 hybrids, amphiploids, BC_1 and advanced generations. The grand mean of the chromosome number in mitosis was 2n=5x=35 in the F_1 hybrids, 2n=10x=70 in the amphiploids and 56 in the BC_1 generation. These data fitted the expected values. The number of bivalents indicated the degree of homology between the hexaploid wheat genome (ABD) and the *T. timopheevii* genome (AG) in the F_1 hybrids. More than 7 bivalents and a number of tri- and quadrivalents were observed, showing the homology of the A genome in both species, and also the partial homology of the B and G genomes. There was a relatively high number of chiasmas per cell, indicating a large amount of association between the genomes of the two species.

Due to the theoretical and practical importance of chromosome behaviour in interspecific and intergeneric hybrids a large number of studies have been carried out and reported. Kihara (1937) analysed the chromosome associations of triploid hybrids involving cultivated *Triticum* spp. obtained by crossing *T. monococcum* with tetraploid *T. turgidum or T. timopheevii*, and by crossing *T. turgidum* or *T. timopheevii* with wild diploid *Triticum* spp. Meiotic chromosome pairing data from triploid hybrids provide a more reliable indication of genomic homologies between parental diploid (2x) and tetraploid (4x) *Triticum* spp. than those from 4x × 4x, 4x × 6x or 2x × 6x combinations.

If, in the parental species, homoeologous genomes are present in addition to completely homologous genomes, the most frequent form of pairing is multivalent (chiefly tri- and quadrivalents). In the cross *T. timopheevii* (n=14, AG) × *T. aestivum* (n=21, ABD) the most frequent chromosome pairing was 10^{II} + 15^{I} or 1^{III} + 9^{II} + 14^{I}. Similar results were also found for ssp. *spelta* × *T. timopheevii*, though other chromosome pairing was also observed. Despite the agreement in the chromosome number, anomalies in cell division can be observed in other interspecific crosses if there are deviations in genome homology. In the F_1 of *T. timopheevii* (AG) × ssp. *carthlicum* (AB), for instance, the most frequent pairing was $12^{II} + 4^{I}$ or $11^{II} + 6^{I}$ (Belea, 1992).

The chromosome configurations of the wheat × *T. timopheevii* amphiploid demonstrate the successful doubling of the genome in the F_1 hybrid. However, as a result of certain irregularities some univalents were also observed. The chromosome pairing data of the plants with 43 and 44 chromosomes did not entirely confirm the assumption that these were monosomic and disomic addition lines. In a monosomic addition line the expected pairing would be one univalent and 21 bivalent, while the observed data showed 19 bivalents, two univalents and one trivalent, showing that this line might contain two alien chromosomes. In plants with 44 chromosomes the expected pairing data of a disomic addition line would be 22 bivalents, but in the present experiments two univalents and 3.6 univalents, respectively, were observed in the two lines. Further studies are required to identify which chromosomes are present in these lines, as it may be that two different G chromosomes have been incorporated, rather than a pair of alien chromosomes.

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ECONOMIC EVALUATION OF PUDDLING METHODS AND WEED CONTROL PRACTICES IN A TRANSPLANTED LOWLAND RICE–RICE CROPPING SYSTEM

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A field experiment was conducted in the wetlands of Tamil Nadu Agricultural University, Coimbatore to study the interactive effects of puddling methods and weed management practices on weeds and on the growth and yield of two rice crops grown in sequence during the dry and wet seasons of 1996–97. Puddling with a tractor-drawn cage wheel restricted the weed growth and resulted in higher grain yields of 5.23 t ha^{-1} and 4.80 t ha^{-1} in the dry and wet seasons, respectively, compared to conventional puddling with a bullock-drawn iron plough (4.97 and 4.65 t ha^{-1} for the respective seasons). The increase in grain yield with cage wheel puddling resulted in a higher benefit:cost (B:C) ratio, especially in the dry season (2.42). The non-chemical, traditional method of manual weeding twice (MW) controlled the weeds effectively, but was not cost-effective. Although allelopathic weed suppression was expected from the planting of parthenium (where initial manual weeding at 20 DAT was skipped), the weed biomass was somewhat greater compared to the other weed control methods. However, the grain yield was higher when parthenium was sown in both the seasons (5.77 and 5.33 t ha^{1} for dry and wet seasons, respectively) compared to 5.53 and 5.12 t ha^{-1} with manual weeding twice and 5.43 and 5.19 t ha^{-1} with butachlor 1.25 kg ha^{-1} + MW in the respective seasons. The increase in grain yield with parthenium application made it possible to achieve better B:C ratios of 2.59 and 2.40 in the dry and wet seasons, respectively.

Key words: weed control, puddling methods, parthenium, rice yield

Introduction

Weeds growing in association with a rice crop reduce the vegetative potential of the crop, ultimately resulting in substantial yield losses (Kim et al., 1979). Yield reductions due to weed competition vary to a great extent depending on the interaction of factors such as cultivars, weed species, population density, the time of emergence of the weeds and the duration of competition (Chisaka, 1977). Weeds vary in their growth habit and life cycle. Therefore, no single method gives continuous and effective weed control.

Puddling, although a capital- and energy-intensive process, is practised to achieve weed control and to increase water and nutrient use efficiencies. The degree of puddling depends on the type of implement used and the intensity of puddling (Taneja and Patnaik, 1962; Kokubun et al., 1969). Rotary implements are expected to give better puddling than ploughs (Koeings, 1963). Puddling, a major method of land preparation, reduced the number of weeds (Reddy and Hukkeri, 1979) and Kuipers (1975) considered tillage as the most important

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weed control practice where chemical weed control was not practised. Tyan (1979) reported 52% control of weeds with tillage alone, whereas tillage combined with the pre-emergence application of a herbicide increased weed control to 97%.

As a means of biological weed control, the allelopathic phenomenon has been exploited to a great extent in recent years. *Parthenium hysterophorus* Linn. is a weed with a history of being an agricultural pest. This plant adversely affects other nearby vegetation by extruding chemicals which inhibit the growth of these other plants. The aqueous extraction of *P. hysterophorus* has yielded parthenin, coronopilin, and two new sesquiterpene lactons closely related in structure to the former compounds. These compounds are believed to be responsible, at least in part, for the allelopathic properties of this plant (Thompson, 1985). The usefulness of parthenium for weed control and as green manure in rice fields has been reported (Purushothaman et al., 1990; Son, 1995). The extent to which these phytoinhibitors are active in the natural environment, however, is still to be established (Adkins and Sowerby, 1996). The objectives of the study were:

a) to compare different methods of puddling for weed control in transplanted rice,
b) to determine whether the composition of the weed flora was altered by different puddling methods, and
c) to measure the effect of puddling methods and weed control treatments (manual, chemical and allelopathic) on crop yield.

Materials and methods

The experiment was conducted on a clay loam soil at the wetland farm of Tamil Nadu Agricultural University. Two transplanted rice crops were grown in sequence in the dry and wet seasons of 1996–97. The available nutrient status of the soil was characterised by low N and P and high K contents. The first crop was planted on June 25th and the second on October 27th. A split plot design with three replicates was used, with the methods of puddling as the main plots and the weed control treatments as the subplots. The two methods of puddling included the use of an animal-drawn wetland iron plough and a tractor-drawn cage wheel, while the weed control treatments consisted of a chemical method (butachlor 1.25 kg + 2,4-D 0.50 kg ha^{-1}), a combination method (butachlor 1.25 kg ha^{-1} + manual weeding - MW), the allelopathic approach (incorporation of fresh parthenium plants 5 t ha^{-1} + MW) and a non-chemical method (MW twice), together with an unweeded control.

The experimental field received uniform dry ploughing with a tractor-drawn cultivator (2 passes) during mid-May, utilizing the moisture from summer rainfall. The field was equally divided and the puddling treatment was carried out in the respective main plot using a tractor-drawn cage wheel or an animal-drawn wetland iron plough. In subplots receiving herbicide, the calculated quantity of chemical was mixed with sand and broadcast uniformly on the plots 3 days after transplanting (DAT). Freshly collected parthenium plants were incorporated in the puddle soil 7-10 days before transplanting the crop. For both crops, 100:50:50 kg N, P_2O_5 and K_2O ha^{-1} was applied as urea, single super phosphate and muriate of potash, respectively. All the P_2O_5 and K_2O and 50% of the N was applied basally. The remaining N was applied in two equal splits at tillering and at the panicle initiation stage of the crop. Twenty-five-day-old seedlings of cv. ADT 36 and ADT 38 were transplanted at a spacing of 15 × 10 cm (dry season) and 20 × 10 cm (wet season), respectively, with two to four seedlings per hill.

The effect of tillage methods on the weed population at 50 DAT, was assessed based on the weed population in the unweeded control plots of the respective puddling treatment. Weed samples were taken from two 0.25 m^2 quadrates per plot at 25 and 50 DAT. The samples were bulked, separated to species, dried at 80°C for 48 hours, and then weighed. The crop harvest area was 10 m^2 and the yields were expressed in t ha^{-1} at 14% moisture content. The nutrient content of the parthenium plants used in the study was analysed using standard plant analysis methods.

Results and discussion

Weed flora

Prior to the start of land preparation, the different weed species present in the experimental field were: *Echinochloa crus-galli* (L) Beauv., *Leptochloa chinensis* (L.) Nees, *Cyperus difformis* L., *Marsilea quadrifolia* L. and *Monochoria vaginalis* (Burm. f.) Pers.

Effect of treatments on weeds

The data presented in Table 1 on the relative weed density recorded in the unweeded plot to assess the effect of tillage methods on the weed population reveal that the grassy weed population was reduced by 5 to 6% and 2 to 3% during the dry and wet seasons, respectively, in both the puddling methods compared to the initial population. The density of *Cyperus difformis* increased to 17.3 and 13.4% under iron plough puddling from an initial density of 12.4 and 7.2% during the dry and wet seasons, respectively, whereas tractor cage wheel puddling reduced the sedge population, especially in the dry season, to 8.6%. In contrast to this, the density of dicots remained unaffected after iron plough puddling, while cage wheel puddling increased the dicot weeds to 60.8 and 66.4% from 51.4 and 63.3% during the dry and wet seasons, respectively. These results are in accordance with the recent findings of Chinnamuthu (1997) in wet-seeded rice. The lower weed biomass recorded at 25 and 50 DAT during both the seasons, convincingly proved the superiority of tractor cage wheel puddling in containing weed growth (Table 2). According to Majid et al. (1988), land preparation with rotary implements proved to be a superior technique among tillage operations under wet conditions, as the weed stand was minimised.

The data on weed dry weight at 25 DAT reveal that the parthenium-incorporated plots and treatments which were manually weeded twice (which had not received any weed control measures at that stage) recorded significantly higher weed biomass compared to treatments in which the pre-emergence herbicide butachlor was applied. It is interesting to note that the weed growth was promoted by parthenium as against the expected suppression of weeds by its allelopathic effect. The dry weight of weeds at 25 DAT in plots planted with parthenium was 72.1 g m^{-2} compared to 53.0 g m^{-2} in the unweeded control plot in the dry season. The weed weights recorded at 50 DAT in the different weed control treatments project a different picture. The control of weeds by two manual weedings and the application of butachlor + MW significantly reduced the weed dry weight in both the seasons compared to the application of a herbicide mixture (butachlor + 2,4-D) without follow-up MW and to the planting of parthenium + MW (Table 2).

Table 1
Relative weed density (%) at 50 DAT in unweeded plots to assess the effect of puddling on the weed population in dry and wet seasons, 1996–97

Weed species	Relative weed density (%)					
	Initial		50 DAT			
			Wetland iron plough puddling		Tractor-drawn cage wheel puddling	
	dry	wet	dry	wet	dry	wet
Echinochloa crus-galli	27.8	18.2	21.4	20.2	22.4	17.3
Leptochloa chinensis	8.4	11.3	10.2	10.1	8.2	8.9
Total grasses	**36.2**	**29.5**	**31.6**	**30.3**	**30.6**	**26.2**
Cyperus difformis	12.8	7.2	17.3	13.4	8.6	7.4
Total sedges	**12.8**	**7.2**	**17.3**	**13.4**	**8.6**	**7.4**
Marsilia quardrifolia	38.9	42.4	34.3	38.3	43.4	48.5
Monochoria vaginalis	6.9	8.2	7.9	11.2	8.2	9.7
Others	5.6	12.7	8.9	66.8	9.2	8.2
Total broad leaves	**51.4**	**63.3**	**51.1**	**56.3**	**60.8**	**66.4**

Table 2
Weed dry weight and panicles of transplanted rice as affected by puddling and weed control methods in dry and wet seasons, 1996–97

Treatment	Weed dry weight (g m^{-2})				Panicles (m^{-2})	
	25 DAT		50 DAT			
	dry	wet	dry	wet	dry	wet
Puddling methods						
Wetland iron plough puddling	47.8	40.9	59.4	32.8	403	361
Tractor-drawn cage wheel puddling	37.8	32.2	47.8	26.0	439	375
$CD_{0.05}$	5.67	5.35	3.21	4.42	19	14
Weed management methods						
Butachlor 1.25 + 2,4-D 0.5 kg ha^{-1}	17.6	14.0	36.8	25.1	443	384
Butachlor 1.25 kg ha^{-1} + MW	18.9	13.5	23.9	16.4	473	394
Pathenium 5 t ha^{-1} + MW	72.1	50.0	29.7	21.5	473	394
Manual weeding (MW) twice	51.3	52.7	15.6	12.6	457	392
Unweeded control	53.0	55.6	162.0	71.2	296	306
$CD_{0.05}$	20.52	8.47	9.25	6.99	18	22

Effect of treatments on crop and yield

The substantial reduction in weed growth and the enhanced crop growth achieved with tractor cage wheel puddling resulted in greater panicle production (439 and 375 m^{-2} during the dry and wet seasons, respectively) compared to iron plough puddling (403 and 403 m^{-2}). Due to this positive effect, the grain yield was enhanced by 0.26 t ha^{-1} in the dry season and by 0.15 t ha^{-1} in the wet season after cage wheel puddling treatment compared with grain yields of 4.97 and 4.65 t ha^{-1} in the dry and wet seasons, respectively, with iron plough puddling (Table 3).

Table 3
Grain yield and economics of transplanted rice as influenced by puddling and weed management methods in dry and wet seasons, 1996–97

Treatment	Grain yield (t ha^{-1})		Benefit:cost ratio		Weed index (%)	
	dry	wet	dry	wet	dry	wet
Puddling methods						
Wetland iron plough puddling	4.97	4.65	2.36	2.22	–	–
Tractor-drawn cage wheel puddling	5.23	4.80	2.42	2.23	–	–
$CD_{0.05}$	0.12	NS	–	–	–	–
Weed management method						
Butachlor 1.25 + 2,4-D 0.5 kg ha^{-1}	5.27	4.81	2.51	2.31	8.7	11.1
Butachor 1.25 kg ha^{-1} + MW	5.43	5.19	2.48	2.38	5.9	4.1
Parthenium 5 t ha^{-1} + MW	5.77	5.33	2.59	2.40	–	–
Manual weeding (MW) twice	5.53	5.12	2.46	2.29	4.1	5.8
Unweeded control	3.52	3.18	1.92	1.79	39.0	41.2
$CD_{0.05}$	0.29	0.25	–	–	–	–

*NS = non significant

Kandasamy and Krishnakumar (1997) also recorded less weed growth, more panicles/unit area and higher grain yield in transplanted lowland rice after tractor cage wheel puddling compared to conventional bose plough puddling.

The weed control treatments indicate that although parthenium incorporation favoured weed growth to a limited extent, consequent on its supply of plant nutrients, it enhanced the crop growth to a larger extent, as evident from the maximum panicle and grain production (473 and 394; 5.77 and 5.33 t ha^{-1}, respectively, for the dry and wet seasons) due to the supplementation of a substantial quantity of plant nutrients. The estimated macronutrient content of the parthenium plants used in the study on a wet basis was 0.81, 0.12 and 0.98% N, P and K, respectively. The green manuring effect of parthenium was earlier reported by many workers (Purushothaman et al., 1990; Kohli and Daizy, 1994; Johnkutty, 1996). The data in Table 3 show that the widely practised weed control practice of manual weeding twice and of butachlor + MW also led to comparable yields in both the seasons. However, chemical weeding (butachlor + 2,4-D alone without MW) did not control the weeds effectively, and thus caused 8.7 and 11.1% yield reduction in the dry and wet seasons, respectively. Due to unchecked weed growth, the grain yield reduction was 39% in the dry season and 41.2% in the wet season in the control plot.

The economic analysis revealed that, though the cost of cultivation was marginally greater with parthenium incorporation, as a result of the nutrient contribution the increase in grain yield led to higher B:C ratios of 2.59 and 2.40 in the dry and wet seasons as compared to the next best treatment of butachlor + MW (2.48 and 2.38), which is comparable with MW twice (2.46 and 2.29) for the two seasons, respectively.

The results of the present study reveal that the productivity of lowland rice is influenced to a great extent by puddling methods and weed control practices. Rotary puddling with a tractor cage wheel restricted weed growth and enhanced

the crop yield as well as improving the economic returns compared to traditional iron plough puddling. Manual weeding, though effective, is labour-intensive and not economical compared to the combined method of butachlor + MW. However, in the context of environmental concern and questions about chemical residues and pollution, weed suppression by non-chemical and/or cultural methods seems to be the need of the future. In the present study, the incorporation of parthenium enhanced crop growth by virtue of enriching the soil with its nutrient contribution. It competitively suppressed weed growth, encouraging its use as an alternative non-chemical method of successful weed suppression. Thus, tractor cage wheel puddling combined with the application of parthenium + MW could effectively reduce weed growth and enhance the yield and economic returns of the transplanted lowland rice-rice cropping system.

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BEHAVIOURAL DEVELOPMENT OF HOLSTEIN-FRIESIAN COWS AND CALVES

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The behavioural development of Holstein-Friesian cows and calves in different housing systems was examined. There were no significant differences in calving ease between cows housed with different areas per cow and in groups or individually. 32–45% of the cows housed in groups separated themselves from the group just before calving. In the cow-calf relationship, the sensitive period in the cow was expressed for 6 hours after the first calving and increased following the next calving. This behaviour of the cow could help the new-born calf to obtain colostrum in time.

The early sensitive period of the calves developed in the first 10 days after birth. Calves that had more human care during this period became easier to manage.

Key words: heifers, cows, calves, development, calving, behaviour, sensitive period, critical period, imprinting

Introduction

The traditional calving system, where new-born calves were immediately separated from their mothers, facilitated careful calf management. Nowadays this traditional system develops in a more natural way. The practice of calving in boxes, in group housing or on the pasture is spreading world-wide and is becoming more and more popular among breeders. New-born calves often stay with their mothers for a short time.

Although this change of system has the great advantage of being cost-saving, it carries the risk that the calves will be neglected. A new practice must be developed to make a reasonable compromise between these contradictory facts.

One of the questions which must be clarified is the influence exerted by the housing of calving cows on the initial development of the calves. If the calf is to be housed with its mother it will be necessary to understand the development of the mother-calf relationship.

The first one- or two-week period is a critical stage in the life of the calf in terms of animal hygiene, but the early colostral feeding of the calves is also very important in this stage. However, very little knowledge is available on the maternal effect, a substantial environmental factor.

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According to some authors (Fraser, 1968; Finger and Brummer, 1969; Craig, 1981; Kaphengst, 1985) grouped pregnant cows showed isolating behaviour just before calving. Kaphengst (1985) reported on the positive influence of milking cows on the vitality of their calves. Sándor (1990) found that the motional ability of calves was better when kept with their mothers than without mothers. A much more natural selection can be found in beef cattle in respect of the mother-calf relation (Houpt and Wolski, 1982). In the mother-calf relation the maternal influence develops earlier and is followed by the reaction of the calf. This is supported by observations on rare breeds kept in their natural environment (Hungarian Grey, Camargue, Maremann), which suggested that if the mother visited her separated calf in the first few days, the calf gradually identified the mother during the separation period (Schloeth, 1961; Dávid, 1984; Vitale et al., 1986). Vince et al. (1985) also indicated the eye-capturing effect of motion in the development of sensitive relationships.

It is not quite clear when and how the social relations of calves change after birth. Edwards (1983) found in his experiment that 33% of new-born calves suckled on cows other than their mothers; in other words, they did not yet recognise their mothers. A similar conclusion was drawn by Arnold and Dudzinski (1978). This imprinting behaviour is identical with the behaviour called first contact by Scott (1978). Pytloun et al. (1986) observed that calves keeping only loose contact with their mothers suckled rarely and their body weight gains were lower. This drop in their performance was not caused by a low IgG level. According to Metz (1987) it is advantageous if calves are kept with their mothers for a short time after birth, because their initial growth and motional ability will be better. The weaning time of new-born calves is also important for animal hygiene, as demonstrated by an experiment carried out by Heinrichs et al. (1987a) on 329 farms, where 44% of the farmers wean calves immediately to prevent infections and 33.7% keep the calf with the mother for 2–4 days.

The necessary individual care is almost entirely lacking in the present practice of calf rearing, which could lead to severe animal health problems. Deaths among calves often exceed 10% and it has been found in Hungary that a considerable portion of the heifers and cows are difficult to handle and are susceptible to stress. The effect of individual care has not been examined in cattle, but results are available for other species. Hemsworth et al. (1986a, b) found that care improved the degree of approach reactions in young pigs. Heird et al. (1986) established that the increased care of foals decreased their restlessness and increased their performance later.

The objective of this study was to determine

– whether housing conditions had any considerable influence on calving ease;

– to what degree cows separated themselves from their herdmates in the case of grouped calvings and what the limit of the group size was in such cases;

– how and when imprinting behaviour developed in calves (Wiepkema, 1991);

– whether surplus human care could improve the manageability of calves.

Materials and methods

Experiments were carried out in a Holstein-Friesian herd of 650 cows. The behaviour of cows kept in stalls of different sizes was observed before and after calving, individually and in groups. The ratio of cows which separated themselves from the group, and the duration and ease of calvings were determined. Nearly 80% of calvings took place in the period between January and April. Data analyses were done only for calvings without complications. The same person assisted at the calvings throughout the experiment.

The time spent on calf care by the cow was determined after the first and second calving, and the time which passed until the calf could stand up, suckle, defecate and urinate was also established. In the case of grouped calvings the frequency of interactions between the cows over a 24-hour period was recorded in a small group of 13 cows and in a bigger one of 28 cows. In this case the area was 15 m^2 per cow.

To investigate the mother-calf relation, calves were weaned at the 7th hour after birth in group 1, while they were left with their mothers for one day in group 2 and for three days in group 3. Data on the calf care time of the cows excluded time spent on suckling. The maternal effect was calculated from the calf care time of the cow. The frequency of offensive behaviour by cows caring for their calves to other herdmates was also recorded. In this experiment the cows were kept with their calves for one day in group 1 and for two days in group 2.

The data were statistically analysed. Differences between the groups were determined with the t-test and correlations between behavioural patterns were also established. The exploratory patterns described by Wiepkema et al. (1987) and Kerr and Wood-Gush (1987) were used for the indication of stress behaviour.

When investigating the sensitive behaviour of the calves, the frequency of approaching other calves was measured. The calves in this experiment were kept in an indoor enclosed area of 6 × 8 m, close to another calf with unrestricted movement. The frequency with which the experimental calves made approaching movements was measured. The end of the sensitive period was determined using the ratio of calves showing fear (Hinde, 1970).

In order to determine the effect of carer activity, the behavioural reactions of calf groups of 25 animals were observed for 1, 3, 7 and 10 days when the calves were given 20 minutes of intensive surplus hand contact daily (for 7 days) on the head and neck by the same carer. The calves were separated from their mothers immediately after birth. Following the method of Hemsworth et al. (1986a, b) the frequency with which the experimental calves approached strangers was observed at 50 days of age, and the body weight of these calves was also measured. Calves showing approaching behaviour were given 2 points, while those which moved away scored 1 point. Finally the mean of these values was calculated for each group. Therefore, if this manageability index was 2 it indicated the tame behaviour of the calves, while if it was less it indicated restless behaviour.

Results and discussion

On the basis of our observations it may be stated that the management system did not have a significant influence on the calvings of Holstein-Friesian cows (Tables 1 and 2).

Table 1
Data on calvings in different housing systems

Housing system	Isolated			In groups		
Designation of groups	1	2	3	1	2	3
Area/cow (m^2)	9.20	7.00	6.00	62.00	35.00	15.00
Total number of cows (n)	28	22	27	25	31	29
Cow separation before calving						
n	–	–	–	8	14	12
%	–	–	–	32	45.16	41.37
Calving unassisted						
n	21	18	24	19	23	21
%	75.00	81.81	88.88	76.00	74.19	72.41
Calving assisted						
n	7	4	3	6	8	8
%	25.00	19.14	11.12	24.00	25.80	27.59
Mean calving time (minutes)						
calving unassisted	43.34	59.32	48.17	45.91	39.87	47.35
calving assisted	76.13	83.36	77.10	78.02	88.12	81.00
Calf sex ratio (heifer/bull)	12/16	10/12	17/10	11/14	18/13	15/14

Table 2
Behaviour parameters of cows and their calves

Housing system	Isolated			In groups		
Designation of groups	1	2	3	1	2	3
Area/cow (m^2)	9.20	7.00	6.00	62.00	35.00	15.00
Total number of cows (n)	28	22	27	25	31	29
Calf care time of cows up to suckling$^+$	49.81	37.94**	51.70**	37.40	45.20	56.36
Time until calf first stood up$^+$	64.90	88.04	84.99	88.85	83.03	70.81
Time until calf first suckled$^+$	93.86**	142.49**	101.00	135.38	124.31	106.07
Time until calf first defecated$^+$	419.13	520.42	440.56	513.28**	440.79	387.41**
Time until calf first urinated$^+$	458.21**	572.40**	474.19	647.20**	488.50	412.23**

$^+$minutes

79.7% of calvings took place without human help. The increased calving room per one cow (9.2 m^2 for an individual and 62.0 m^2 for a group) did not improve the productivity of calvings. There was no significant difference between the groups. The frequency with which the cows disturbed each other was less in the small group (186) and almost twice as much in the bigger group (289). This difference was significant at the $P<0.01$ level. However, the aggressive behaviour of the cows may be influenced by their temperament.

32–45% of the animals kept in groups showed separating behaviour before calving. The calves of cows which calved in isolation rarely tried to

suckle on other cows. This 'foreign suckling' occurred in 24.6% of the calves over the mean of the three groups. Cows which calved simultaneously allowed foreign calves to suckle for a few hours following calving. There was no significant correlation between the body weight of the calves at birth and the vitality of the new-born calves, either within the observed groups or when comparing the two calving methods.

Maternal influence, however, characterised by the calf care time of the cows, had an impact on the vitality of the calves. Negative correlations were found between the calf care time of the cow and the time which passed before the calf first stood up. When the cows calved individually in isolation this correlation was –0.52 in the first group, –0.67 in the second group and –0.49 in the third group. The correlations between calf care time and the time until the calf first suckled (in the same order as before) were –0.58, –0.69 and –0.51, respectively. The more time the cow spent on licking her calf, the earlier the calf stood up and suckled.

In the case of group calvings there was only a significant correlation (–0.71) between the calf care time of the cows and the time until the calf first stood up in the group kept on the smallest area (15 m^2). The correlations between calf care time and the time until the calf first suckled were (in the same order) –0.68, –0.41 and –0.56, respectively.

Figures 1 and 2 demonstrate changes in the first time when calves kept with their mothers stood up and suckled, both of which are very important in providing the calves with colostral immunity. Only 45% (69 animals) of the calves kept in the group calving experiments stood up in the first hour following birth. Therefore, only about 51% (77 animals) of the new-born calves in this group calving experiment obtained a sufficient amount of colostrum in the first two hours. New-born female calves stood up after birth and started to suckle a little earlier than male calves, but the difference was not significant.

Therefore, the mother has a positive effect on the colostrum uptake of the calf, but this is not sufficient for an adequate colostrum supply to the calves. A switchover to grouped calving alone cannot solve the safe colostrum supply of the calves.

The results of further experiments support the assumption that the time spent by the cow in caring for her calf changed considerably on the first day after calving (Table 3). The mother-calf relation develops gradually. The cow licks and smells her calf. Tactile and smelling stimuli are essential in the improvement of the mother-calf relation. The mother marks her calf through her care of it, so the real purpose of the procedure is identification. The care time of the cows varied in different periods and was greater in subsequent calvings.

The cows spent relatively little time on the care of their calves in the first two hours after calving. The average care time showed a sudden increase in the next period of 2–6 hours after calving, decreasing again during the period 6–24 hours after calving.

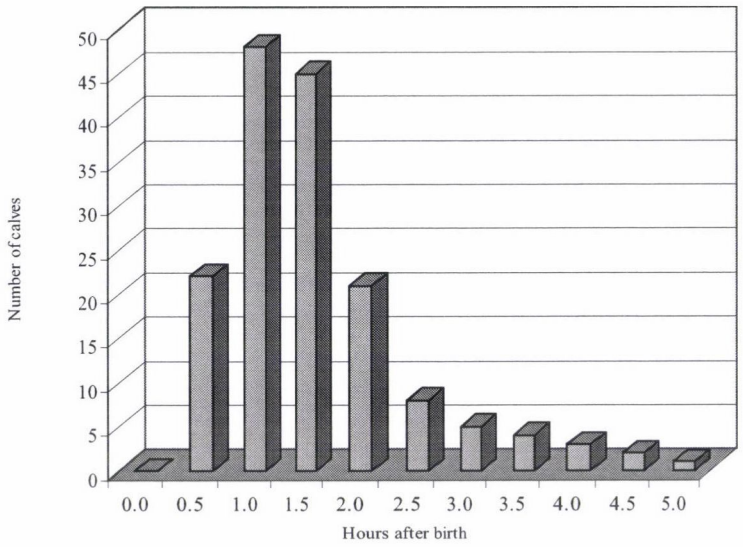

Fig. 1. Distribution diagram for the time which passed before the calves first stood up*
*Total of cow-calf groups, without isolation, n = 152

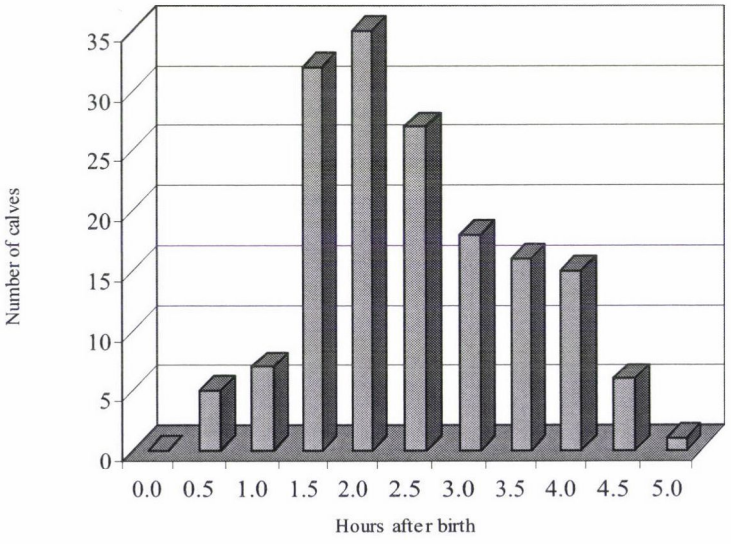

Fig. 2. Distribution diagram for the time which passed before the calves first suckled*
*Total of cow-calf groups, without isolation, n = 152

On the basis of the data in Table 3, the differences between the first two periods in Group 2 (when the calves remained with the cows for one day) were significant at the $P<0.01$ level, while in the case of cows caring for their second calf and housed similarly to Group 2, the differences were significant at the $P<0.001$ level. (The designations of these two groups are identical in Tables 3 and 4). The caring behaviour of the cows did not change substantially after one day had passed. Therefore, it can be concluded that the caring behaviour of the cows was most intensive during the period 2 to 6 hours after calving. In the case of the second calving, a year later, the maternal behaviour was more intensive during the first three days after calving ($P<0.05$) than a year before. The cows spent more time on their second calves.

Observations proved that early experience obtained in the period just following calving was very important. In the groups of cows where the calves were kept with their mothers for one or three days, the experience gained by the cow in the period 2–6 hours after the first calving lasted for the period of the second calving. In this case the increase found in calf care time was significant at the $P<0.001$ level.

Cows separated from their calves immediately after the first calving spent less time on their second calves than their "more experienced" herdmates. The period 2–6 hours after calving was the critical one, during which cows with little experience spent significantly ($P<0.001$) less time on calf care than cows kept with their first calves for one day (Table 4).

Table 3
Distribution of the care time spent by the cows on the first calf

Period in hours after calving	Groups*	1	2	3
	n	35	34	24
	Care time			
0–2	minutes	–	12.62	26.01
	% of 24 hours	–	0.87	1.80
	cv	–	20.60	14.30
2–6	minutes	–	29.67	37.83
	% of 24 hours	–	2.12	2.62
	cv	–	13.50	12.90
6–24	minutes	–	20.58	30.81
	% of 24 hours	–	1.42	2.13
	cv	–	23.30	15.90
24–72	minutes	–	–	34.17
	% of 24 hours	–	–	2.37
	cv	–	–	20.50

*Group 1: Calf separated from the cow after 7 hours
Group 2: Calf with the mother for one day
Group 3: Calf with the mother for three days

Provenza and Balph (1987) reported a similar selective and active learning process in the sensitive period. Thus, the calf care "performance" of the cows can be improved during subsequent calvings and during the short nursing periods. This behaviour has a positive effect on the early standing up, suckling and defecation of the calves. Maternal behaviour is most intensive in the period 2–6 hours after calving. The variance of this behaviour decreases with age and particularly during the relevant period.

The variance values were relatively high in the first group in Table 4 (cows with little experience). In this group, the sensitive period (2–6 hours following calving) was extended to the next period (more than 6 hours after calving).

Table 4
Distribution of care time spent by the cows on the second calf

Period in hours after calving	Groups*	1	2	3
	n	35	34	34
	Care time			
0–2	minutes	17.11	23.74	26.01
	% of 24 hours	1.18	1.64	1.80
	cv	24.90	14.40	14.30
2–6	minutes	28.10	49.23	37.83
	% of 24 hours	1.95	3.42	2.62
	cv	18.40	11.00	12.90
6–24	minutes	22.71	24.04	30.81
	% of 24 hours	1.57	1.66	2.13
	cv	15.40	18.20	15.90
24–72	minutes	21.18	28.61	34.17
	% of 24 hours	1.47	1.98	2.37
	cv	21.30	17.90	20.50

*Group 1: First calf separated from the cow after 7 hours
Group 2: First calf with the mother for one day
Group 3: First calf with the mother for three days

Figure 3 demonstrates the early sensitive period of maternal behaviour. In optimal cases, cows can identify their calves 6–7 hours after calving. This identification is indicated by the increasing frequency of aversive movements of the cows towards other cows and foreign calves. Active, aggressive behaviour is typical of the Hungarian Grey breed, but is very rarely observed in modern dairy breeds. The best time for calf identification by the cow is the period 2–6 hours after calving. The shortness of this optimal period is also emphasised by Kilgour (1975).

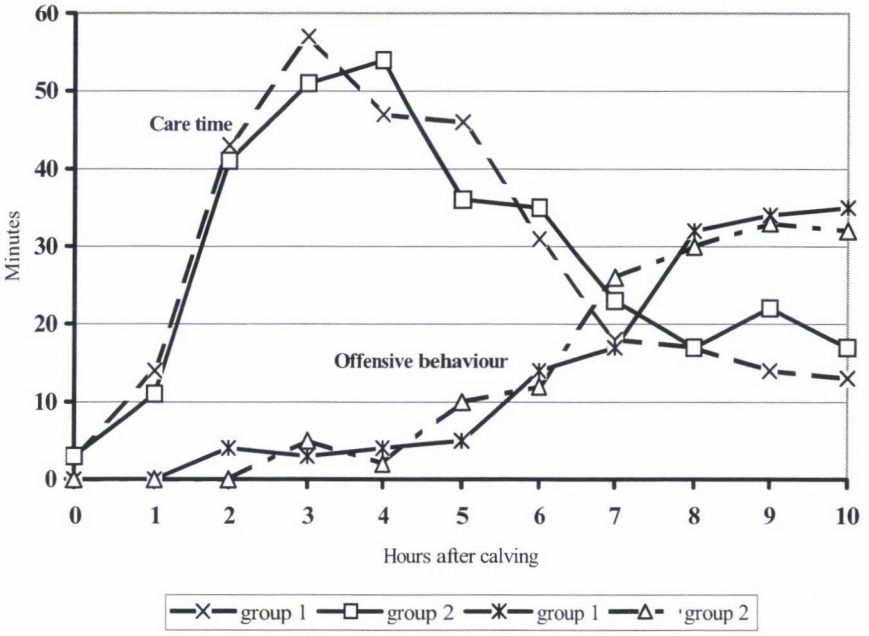

Fig. 3. Time spent by the cows in caring for their new-born calves, and offensive behaviour
Group 1 = Calf with the mother for 1 day, Group 2 = Calf with the mother for 3 days

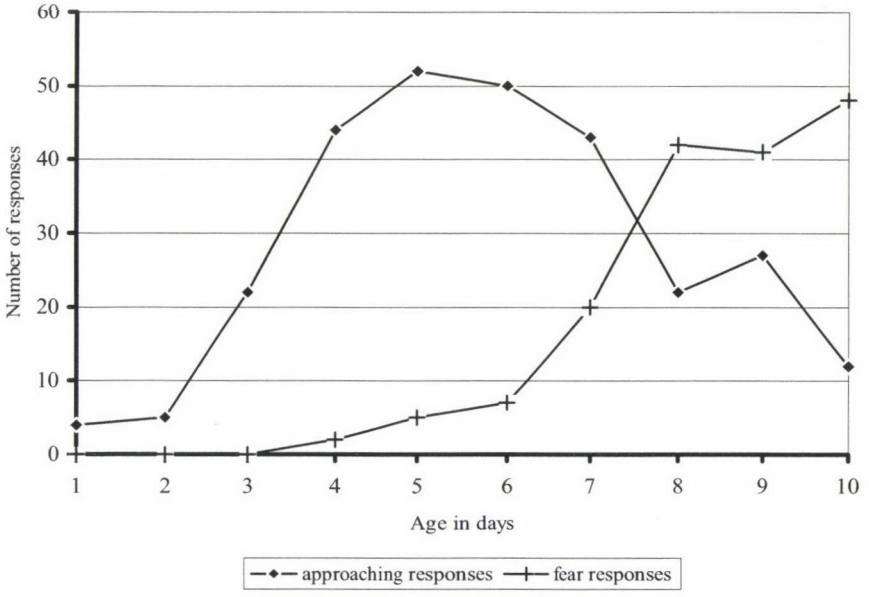

Fig. 4a. Approach and fear responses of the calves during the first 10 days of life
(The calves were kept with their mothers for 1 day after birth)

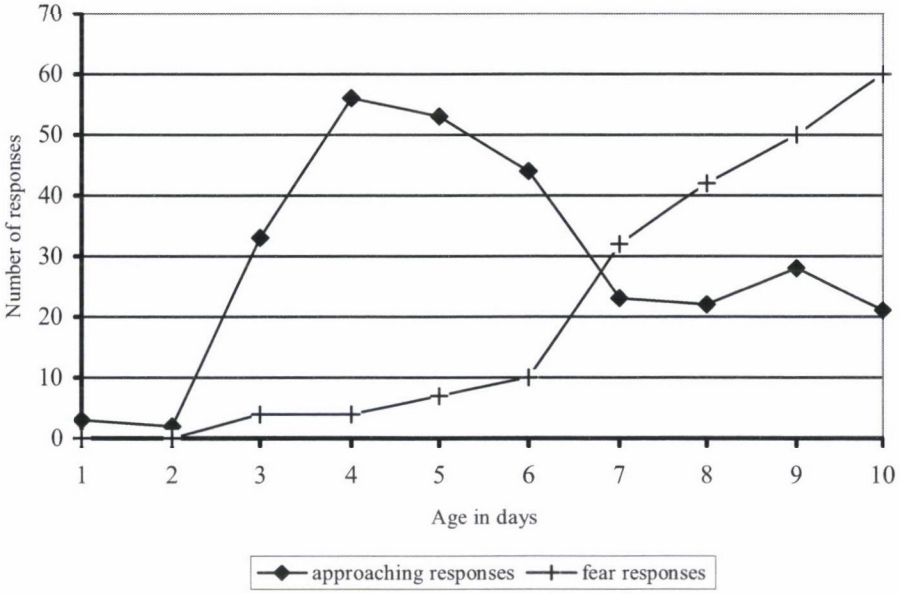

Fig. 4b. Approach and fear responses of the calves during the first 10 days of life (The calves were kept with their mothers for 3 days after birth)

Figure 4a,b shows that calves were particularly responsive towards persons and animals on the move from 3–4 days of life to 7–8 days. Inquisitiveness towards unknown objects, persons and animals was gradually blocked by fear reactions, which increased noticeably from the 3rd–4th days. The attachment of calves to their mothers was strengthened from the 4th–7th days of age.

The development of the mother-calf relation (the strong attachment between cows and calves) is well demonstrated by the fact that cows of rare breeds generally "lead out" their calves on the 3rd–4th days. The time when the attachment of the calves to their mothers develops to the greatest extent is generally during the sensitive period, on the 4th–6th days. Fraser (1974) also stated that calves more than three days old could hardly be separated from their mothers. A comparison of Figures 4a and 4b suggests that this attachment of the calves may take place even without the permanent presence of the cow. However, the process of forming an attachment may be faster when the calf is kept with the cow for several days.

Figure 5 shows that calves separated early from their mothers and kept in individual pens are more restless than those kept with their mothers for some days. The exploratory behaviour of the calves is in direct proportion to their degree of restlessness.

The results suggest that calves should be kept with the cow for a certain period after birth. The critical maternal response develops on the first day after calving, the optimal time for this being 4–6 hours after calving.

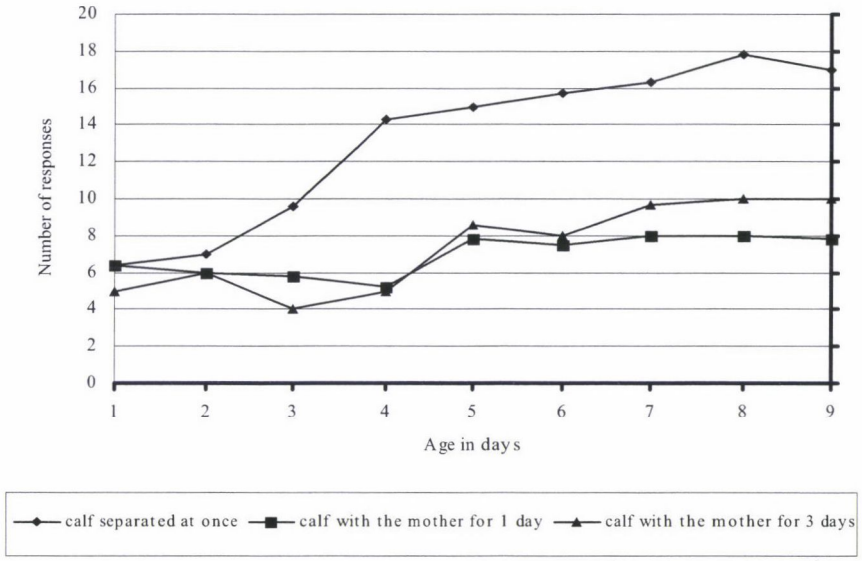

Fig. 5. Exploration frequency of calves raised using different methods

Observations show that cows which nursed their first calves for 1–3 days spent more time on caring for their second calves than those separated from their first calves immediately after calving. Therefore, it can be concluded that even a short nursing period could improve the calf care ability of the cows.

In the present experiments the immediate separation of the calves from their mothers resulted in uncertain calf care ability in the cows. The absence of this ability could delay the first suckling and defecation of the calves in the case of group calvings.

Calves weaned at the age of 5–6 days or later have a much greater fear of new, unknown environments than those weaned earlier. Therefore, it is recommended that calves should be separated from the cows by an age of 2–3 days and should be provided with an environment rich in stimuli and human contacts. This statement can be applied to animal health treatments and observations (Price, 1984).

This recommendation is even more important in an outdoor environment with individual pens for the calves. For the full maturation of muscle tone and better early motional ability it is advantageous to keep the calves with their mothers for a few days after birth. The general practice in Hungarian herds is for calves separated early from the cow to be housed in narrow individual pens with slotted floors. This environment is favourable to the development of foot disorders.

The response of calves to additional human care was studied up to one week of age. The results (Table 5) show that the daily surplus human care had a positive effect, to different extents, on the manageability of the calves.

Table 5
Effect of human care on the manageability of calves at different ages

Studied features	Age in days			
	1	3	7	10
Basic care time (cleaning, feeding) (minutes)	5	5	5	5
Surplus care time (minutes)	20	20	20	20
% of 50-day-old calves approaching human	10	52	38	8
Mean body weight of 50-day-old calves (kg)	63.9	65.4	66.2	64.4
Mean manageability index	1.36	1.94	1.98	1.58
n	25	25	25	25
Differences between the age groups (day)	*t-value*		*SD P<0.05*	
1–3	6.829***		0.288	
1–7	3.954***		0.315	
1–10	1.596		non significant	
3–7	2.167*		0.333	
3–10	4.625***		0.321	

Surplus human care time spent on calves between the 3 rd and 7th days of life resulted in good manageability indices on the 50 th day. With one exception the differences between the observed age groups were significant, indicating an improvement in this behaviour. The low, statistically non-significant means of groups aged 1 and 10 days indicate the age limits of this learning process. The frequency with which 3- and 7-day-old calves approached strangers was especially high. The results of the surplus care time experiment show that the manageability of the calves can be improved by surplus care at 1 week of age.

Heinrichs et al. (1987 a, b) also emphasise the essential role of human care in improving the effectiveness of calf management. Tamer than usual behaviour may be connected with lower susceptibility to stress. In practice, calf care can be combined with daily management, herd control and occasional animal health treatments.

The following conclusions can be drawn on the basis of the experiments:

–Housing itself does not have any substantial influence on calving ease.

–In the case of group calvings, some cows must be expected to separate themselves from the herd, so groups of more than 10–12 cows are not recommended. Calving in small groups provides greater peace and quiet for the cows and is safer from the technological point of view.

–The calf care behaviour of cows improves gradually after calving (particularly 2–6 hours after calving). At the end of this period, lasting for a few hours, the cow is able to identify her calf. However, this maternal behaviour improves during subsequent calvings. Maternal effects stimulate the early colostrum intake and vitality of the calves. On the other hand, this maternal behaviour is not adequate in itself, human care is also needed.

–The first sensitive period of the calves develops in the first week of life. Social relations are relatively weak until 2–3 days of age and become stronger on the 4th–7th days. Fear reactions to unknown herdmates, objects or persons intensify during the same period.

–It is useful to keep calves with their mothers for a few days, unless this is impossible for animal health reasons. However, the calves should be weaned and separated from their mothers on the 2nd or 3rd day after birth.

Surplus human care applied after weaning can improve the subsequent manageability of the calves.

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Short communication

GENETIC, PHENOTYPIC AND ENVIRONMENTAL CORRELATIONS BETWEEN TRAITS OF BEEF CATTLE

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In this study paternal half-sib estimates were computed for the genetic, phenotypic and environmental correlations between birth weight, calving difficulty, calf losses and weaning weight, the postweaning gain, age at puberty and rates of oestrus and pregnancy in heifers, and the daily gain, final weight, slaughter and carcass traits of steers.

Data on Hereford and Angus crossbred populations from the USA (with 400–500 animals for each genotype) were analysed with the Least Squares and Maximum Likelihood Computer Program (Harvey, 1990).

The results indicated that in general the genetic correlations were large, the environmental ones low or negative and the phenotypic ones intermediate. The genetic correlation tended to be higher between birth weight and calving difficulty (r_g=0.57), and calving difficulty and calf losses (r_g=0.66). No genetic correlation was found between 400-day weight and puberty age of heifers, but there was a negative environmental correlation (r_e =–0.31).

Key words: calving, birth weight, weaning weight, 400-day weight, 550-day weight, pregnancy rate of heifers, daily gain, slaughter traits of steers

Introduction

The phenotypic variation in any polygenic character is divisible into genetic and environmental components. In the same way, the phenotypic relationship between characters has genetic and environmental parts, i.e. the relationship may be a phenotypic, genetic or environmental correlation.

Phenotypic relationships between the traits of beef cattle are of both theoretical and practical interest. A knowledge of its genetic part, genetic correlation, is needed for the multiple trait evaluation of individuals (Hazel, 1943; Henderson and Quaas, 1976) and for the prediction of correlated responses to selection (Falconer, 1960; Dickerson et al., 1974; Roberts, 1979). On the other hand the environmental part, environmental correlation, may also play an important role in the overall effectiveness of selection for merit. If both correlated characters have high heritabilities the genetic correlation is the more important determinant of phenotypic correlation, but when heritabilities are low the environmental correlation is the major factor.

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The phenotypic and genetic relationships of the most important traits of beef cattle have been widely reported (Carter and Kincaid, 1959; Cundiff et al., 1982, 1986; Dinkel and Brinks, 1973; Hohenboken et al., 1973; Koch et al., 1973; Bourdon and Bush, 1982; MacNeil et al., 1984; etc.). However, there is a paucity of estimates for environmental correlations.

The objective of this study was to estimate from experimental data the phenotypic, genetic and environmental correlations between birth weight, calving difficulty, calf losses and weaning weight, the postweaning gain, age at puberty and rates of oestrus and pregnancy in heifers, and the daily gain, final weight and slaughter and carcass traits of steers.

Materials and methods

For the estimation of the relationships data were taken from a herd of Hereford and Angus cows and from their F_1 progeny sired by Simmental and Charolais bulls. The data were collected and analysed in the USA. The number of animals was between 400–500 for each genotype. The cows were kept on pastures throughout the year and naturally mated in summer. The calves were born in late winter or early spring and weaned in autumn. The heifers were kept on pastures after weaning and observed for the first oestrus. They were then mated with Hereford sires and checked for pregnancy. Bull calves were castrated a couple of days after birth. The steers were fattened in feedlots and slaughtered for experimental purposes.

The statistical analysis of the data was carried out using SAS Version 6, and the Least Squares and Maximum Likelihood Computer Program (Harvey, 1990).

Genetic correlations were computed in each case by simply dividing the "family" covariance component estimate for the two traits by the geometric mean of the two "family" variance component estimates.

Phenotypic and environmental correlations were estimated by the paternal sibs model, where no interaction of the sires with fixed effects were considered.

The models used were as follows:

Phenotypic correlations (r_P):

$$r_{P(hh')} = \frac{\hat{\sigma}_{e(hh')} + \frac{1-NW}{NR1}\hat{\sigma}_{s(hh')}}{\sqrt{\left[\hat{\sigma}^2_{e(h)} + \frac{1-NW}{NR1}\hat{\sigma}^2_{s(h)}\right]\left[\hat{\sigma}^2_{e(h')} + \frac{1-NW}{NR1}\hat{\sigma}^2_{s(h')}\right]}}$$

Environmental correlations (r_E):

$$r_{E(hh')} = \frac{\hat{\sigma}_{e(hh')} + \frac{NW}{NR1}\hat{\sigma}_{s(hh')}}{\sqrt{\left[\hat{\sigma}^2_{e(h)} + \frac{NW}{NR1}\hat{\sigma}^2_{s(h)}\right]\left[\hat{\sigma}^2_{e(h')} + \frac{NW}{NR1}\hat{\sigma}^2_{s(h')}\right]}}$$

where h refers to the h^{th} trait, h' refers to another trait, $\hat{\sigma}^2_s$ is the crossclassified or nested "family" variance component estimate, $\hat{\sigma}^2_e$ the within-family variance component estimate, NW the decimal percentage of additively genetic variance in $\hat{\sigma}^2_e$, and NR1 the decimal percentage of additively genetic variance in $\hat{\sigma}^2_s$.

Results and discussion

The significant correlation values observed between the birth weight, calving difficulty and 400-day weight of heifers with some other traits are summarised in Table 1, and those for the daily gain and final weight of the fattened steers in Table 2.

As can be seen from the tables the genetic correlation tended to be higher between birth weight and calving difficulty, and between calving difficulty and calf losses. No genetic correlation was found between 400-day weight and puberty age, but the environmental correlation was negative. High correlation values were also observed for the daily gain and final weight with the carcass, meat, bone and fat weights, but low values were found for the percentage values.

In general the values obtained for phenotypic and genetic correlations were similar to those previously calculated for very different populations cited in the literature (Carter and Kincaid, 1959; Cundiff et al., 1982, 1986; Dinkel and Bush, 1973; Hohenboken et al., 1973; Koch et al., 1973; Bourdon and Brinks, 1982; MacNeil et al., 1984; etc.). The environmental correlation values were much more similar to the phenotypic correlations than to the genetic ones. As the results show, the sign (negative or positive) of the genetic and environmental correlations is the same for most pairs of traits, though some pairs of traits have the opposite sign (negative and positive) for genetic and environmental correlations. In such cases the phenotypic correlation generally tends towards zero. However, environmental correlations can give useful information about the non-genetic parts of the relationships between the different pairs of traits.

Table 1

Genetic (r_g), phenotypic (r_p) and environmental (r_e) correlations between birth weight, calving difficulty and 400-day weight of heifers

Correlated traits	r_g	r_p	r_e
Birth weight and			
calving difficulty	0.57	0.11	0.03
calving unassisted	–0.55	–0.13	–0.03
caesarian section	0.23	0.04	–0.01
rate of weaned calves	–0.34	0.03	0.11
weaning weight	0.19	0.22	0.23
200-day weight	0.48	0.35	0.31
Calving difficulty and			
early calf mortality	0.60	0.21	0.18
late calf mortality	0.29	0.03	0.01
total calf mortality until weaning	0.66	0.18	0.15
rate of weaned calves	–0.66	–0.18	–0.15
400-day weight of heifers and			
550-day weight	0.91	0.82	0.78
puberty expressed	0.05	0.13	0.18
age at puberty	0.00	–0.16	–0.31
pregnancy rate	–0.04	0.05	0.07

($P<0.005$)

Table 2
Genetic (r_g), phenotypic (r_p) and environmental (r_e) correlations between daily gain and slaughter weight

Correlated traits	r_g	r_p	r_e
Daily gain during fattening and			
final weight	0.97	0.86	0.75
carcass weight	0.92	0.79	0.69
dressing percentage	–0.17	–0.04	0.05
fat thickness	0.18	0.21	0.25
kidney, pelvic and heart fat percentage	0.20	0.07	–0.13
rib eye area	0.30	0.31	0.46
marbling	0.30	0.07	–0.11
meat weight	0.76	0.74	0.72
meat percentage	–0.21	–0.21	–0.22
carcass fat weight	0.53	0.49	0.44
carcass fat percentage	0.23	0.24	0.24
bone weight	0.68	0.63	0.59
bone percentage	–0.17	–0.20	–0.22
Slaughter weight and			
carcass weight	0.96	0.95	0.95
dressing percentage	–0.16	–0.03	0.07
fat thickness	0.13	0.03	0.45
kidney, pelvic and heart fat percentage	0.26	0.12	–0.04
rib eye area	0.30	0.39	0.48
marbling	0.27	0.09	–0.03
meat weight	0.81	0.87	0.93
meat percentage	–0.16	–0.30	–0.47
carcass fat weight	0.51	0.63	0.74
carcass fat percentage	0.18	0.32	0.48
bone weight	0.72	0.75	0.77
bone percentage	–0.16	–0.26	–0.33

($P<0.005$)

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Short communication

EFFECT OF MEDIUM ON THE CALLUS-FORMING CAPACITY OF DIFFERENT POTATO GENOTYPES

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The callus formation of five potato genotypes with different genetic origins was analysed on five different media to select the optimal callusing treatment. The aim of this work was to adapt callusing methods to Hungarian genotypes. Both induction and the rate of callus growth were strongly influenced by genotype and medium, and significant 2nd order interactions were proved statistically. The best undifferentiated growth of friable calli on leaf explants could be observed after 4 weeks on medium containing 0.25 mg l^{-1} kinetin and 5.0 mg l^{-1} 2,4-D.

Key words: *Solanum*, callus induction and growth, genotype, potato

Abbreviations: BA - 6-benzylaminopurine, 2,4-D –2,4-dichlorophenoxyacetic acid, NAA – naphthaleneacetic acid, GA_3 – gibberellic acid

Introduction

Callus and suspension cell cultures can be used to select mutants with improved stress tolerance, which can then be used in breeding programmes (Carputo et al., 1995; Morpurgo, 1991; Wersuhn, 1989). Selection at the level of single cells can be applied to heterozygous and vegetatively propagated species, such as potato (Bajaj, 1987), if the formation of the desired trait by crossing is limited. The response to tissue culture conditions may be different from species to species and from variety to variety. To increase the genetic variability in Hungarian potato clones using *in vitro* selection methods, experiments have been set up to establish a callus culture suitable for the development of cell suspension cultures.

Materials and methods

Plant material

Five potato genotypes were utilised in this study: Desiree, Gülbaba, Réka, Beáta, Rebeka. Gülbaba is of pure *Solanum tuberosum* origin. One of the parents of Beáta and both parents of Rebeka were *Solanum tuberosum* × *Solanum chacoense* hybrids. One of the parents of Réka was a *Solanum tuberosum* × *Solanum chacoense* × *Solanum famatinae* hybrid. The *in vitro* plants were maintained on MS medium (Murashige and Skoog, 1962) supplemented with 3% sucrose and 0.8% agar at 24°C/15°C day/night temperature and 16 h "warm white" light, with monthly subculturing.

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Establishment of callus culture

Leaf explants were taken from 4-week-old *in vitro* plantlets. The apex and bottom part of the leaflets were cut off and thrown away. If the leaf segment was too big it was cut in two parallel to the first cuts. In this way leaf segments of the same size were obtained, each with two cut surfaces. These segments were placed with the abaxial surface in contact with the medium. The explants (10 explants per plate and five plates per treatment) were cultured in 7 cm glass Petri dishes on MS medium (Murashige and Skoog, 1962) supplemented with 3% sucrose and 0.8% agar, while the hormone content was modified in five different ways as follows:

Medium 102 - 0.2 mg l^{-1} kinetin, 0.1 mg l^{-1} GA_3 and 1.0 mg l^{-1} 2,4-D (Dathe and Wersuhn, 1990),
Medium 103 - 0.25 mg l^{-1} kinetin and 5.0 mg l^{-1} 2,4-D (Tavazza et al., 1988),
Medium 104 - 1.0 mg l^{-1} BA and 5.0 mg l^{-1} NAA (Ramulu et al., 1985),
Medium 105 - 2.24 mg l^{-1} BA, 0.2 mg l^{-1} NAA and 10.0 mg l^{-1} GA_3 (Wenzler et al., 1989),
Medium 106 - 2.24 mg l^{-1} BA and 0.2 mg l^{-1} NAA (Higgins et al., 1992).
All the cultures were incubated under the same conditions used for culture maintenance.

Measurements and data analysis

The responses of the genotypes to different media were evaluated after 4 weeks using the following scale: 1 = no response, 2 = a little callus at one end of the leaf segment, 3 = a little callus at both ends of the explants, 4 = strong callus growth, but the surface of the explant is visible, 5 = explant overgrown with callus. Because the present data did not conform to a normal distribution, non-parametric tests were used for the analysis of the data. Differences in callus growth between genotypes and media were analysed by Welch's and Brown-Forsythe's rank tests and the Tukey-Kramer methods (Sachs, 1988) using MiniStat 2.4 computer software.

Results and discussion

The callus induction and growth response of the genotypes, tested on five media, is reported in Table 1. Significant differences were found in callus growth, but callus induction was observable on all the media tested after 4 weeks of culture in all the genotypes examined. The callus growth rate varied from 1.8 to 5.0 depending on the genotype and the medium. Although statistical analysis proved a significant interaction between the media and genotypes at the $p < 0.01$ level, the best medium for callus growth was medium 104. In Rebeka, Réka and Gülbaba there was no significant difference in the callus growth rate between medium 104 and medium 103. However, considerable differences were revealed between the degree of differentiation and the structure of the calli developed on different media. Strong shoot differentiation was obtained on medium 105 in three genotypes (Desiree, Beáta, Rebeka) (data not shown). Although further experiments are necessary, this observation suggests differences in regeneration ability between the genotypes tested. Calli grown on medium 104 often formed roots after 3-4 weeks, as described by Carputo et al. (1995), and the calli were very hard, whereas calli developed on medium 103 hardly ever regenerated and were very friable and vigorous, indicating undifferentiated growth. Because of their structure, the calli grown on medium 103 seemed to be more suitable for the establishment of cell suspension cultures.

Although statistical analysis revealed significant differences between the genotypes in callus growth on all the media, it was generally observable that the poorest rate of callus growth occurred in Rebeka on all the media, while the strongest callus growth was visible in Gülbaba and Réka. Callus growth was significantly different depending on medium and genotype.

Table 1
Effect of medium and genotype on callus induction and growth after 4-week culture under light conditions*

Genotype	Medium				
	102	103	104	105	106
Desiree	2.4±0.4 a, A	3.5±0.5 b, A	5.0±0.1 c, B	3.1±0.5 b, B	2.9±0.2 ab, B
Gülbaba	3.4±0.5 a, B	4.6±0.2 b, B	5.0±0.1 b, B	2.8±0.2 a, B	3.1±0.2 a, B
Réka	3.3±0.5 a, B	4.6±0.3 b, B	5.0±0.0 b, B	3.0±0.2 a, B	2.9±0.2 a, B
Beáta	3.2±0.1 b, B	3.2±0.3 b, A	4.9±0.1 c, B	3.5±0.1 bc, B	2.8±0.1 a, B
Rebeka	1.8±0.5 a, A	3.4±0.5 b, A	4.6±0.3 b, A	2.2±0.4 a, A	2.3±0.3 a, A

Separation of means based upon the robust tests mentioned in the text. Means within rows followed by the same small letter are not significantly different ($p<0.01$) and means within columns followed by the same capital letter are not significantly different ($p<0.01$).
*Data from three independent experiments

The results obtained suggested that callus formation can be induced from all the genotypes after 4 weeks, although they required an optimal hormone content in the medium for callus growth. The optimal combination of media for the given genotype could form the first step in a tissue culture cycle and could thereby be applied in the breeding of potato. Further research is underway in our laboratory to examine the effects of dark (pre)treatment or other temperature conditions on the rate of callus growth.

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Short communication

GENETICS OF LEAF RUST-RESISTANT MUTANT WH 147-LM-1 IN HEXAPLOID WHEAT VARIETY WH 147

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By applying gamma rays, EMS and their combination in hexaploid wheat variety WH 147, a total of 20 mutants (0.0226%) exhibiting complete leaf rust resistance were isolated from segregating M_2 rows. When one of the rust-resistant mutants, WH 147-LM-1 was crossed with the universally susceptible wheat variety Agra Local, the F_2 plants segregated in a 13:3 ratio for resistant to susceptible, suggesting that the mutant character is controlled by one dominant gene and one recessive gene. The F_2 plants derived by crossing the mutant WH 147-LM with seven near-isogenic wheat lines showed segregation for susceptibility, indicating that the mutant character was indeed generated through induced mutations.

Key words: rust-resistant mutants, wheat, genetics

Introduction

The cultivation of resistant cultivars is the cheapest and often the most effective method of avoiding crop losses due to diseases and insect pests. However, the continued evolution of the pathogens and the scarcity of resistance gene sources are the primary limitations to the development and long-term use of resistant cultivars. Plant breeders attempt to employ as many resistance genes as possible in breeding new cultivars and modern agriculture replaces indigenous genetic diversity by a few selected genotypes. This results in allele and gene erosion which could threaten the future of plant breeding. In this connection, induced mutagenesis could be effectively used to obtain additional gene/allele sources for resistance to disease. The present communication reports on a study of the genetics of a leaf rust-resistant mutant induced in a susceptible Indian hexaploid wheat variety WH 147.

Materials and methods

The parent material, hexaploid wheat variety WH 147, was selfed for three successive generations under controlled conditions. Seed obtained from this material was irradiated with three doses of gamma rays (10, 20, 30 kR), three durations of 0.5% EMS (6, 8, 10 h), and various combinations of these (10 kR+10 h; 20 kR+8 h; 30 kR+6h). The individual spikes of all the M_1 plants were bagged, and the seeds obtained were sown as plant progenies to raise the M_2 generation. Under field conditions, M_2 plants were artificially inoculated with a mixture of leaf

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rust races. Inoculation was made three times at weekly intervals to rust build-up. A mixture of rust races was prepared using light mineral oil Mobisol-100 and this was applied with a power sprayer. Rust reactions were recorded 10 days after the third inoculation. Mutant plants completely resistant to leaf rust were isolated from segregating M_2 rows.

One of the leaf rust-resistant mutants, WH 147-LM-1, was crossed with the universally susceptible wheat variety, Agra Local. To confirm its mutant nature, the mutant was also crossed with near-isogenic leaf rust-resistant lines carrying genes *Lr9, Lr18, Lr19, Lr24, Lr25, Lr28* and *Lr29*. The leaf rust pathogens that were used in the study to characterise the mutants include race 108, race 77-1 and race 106. F_2 plants were tested both at the seedling stage in the greenhouse (using races 108, 77-1 and 106) and at the adult stage under field conditions (using race 77-1). The leaf rust intensity on these plants was recorded according to the modified Cobb's scale as described by Peterson et al. (1948).

Results and discussion

The F_2 generation from the cross of WH 147-LM-1 with the universally susceptible wheat variety Agra Local, inoculated with the most virulent leaf rust race 77-1, segregated in a 13:3 ratio of resistant and susceptible plants (1948 resistant plants and 430 susceptible plants; chi-square value 0.6956). In addition all the F_2 plants derived from the crosses of WH 147-LM-1 with the seven near-isogenic leaf rust-resistant lines segregated for susceptibility. Wheat cultivar WH 147 was susceptible (3 to 3+ at the seedling stage and 60S at the adult stage) to all the three leaf rust races used, while the mutant was resistant (0 to 0; in the seedling stage and 'F' - free in the adult plant stage) to the leaf rust races.

The observed segregation (13:3) for the leaf rust reaction to leaf rust race 77-1 in the cross between the mutant and the wheat variety Agra Local suggests the presence of one dominant gene and one recessive gene in the mutant. Leaf rust race 77-1 is virulent to all the known genes for leaf rust resistance from *T. aestivum*. Only the alien genes *Lr9, Lr18, Lr19, Lr24, Lr25, Lr28* and *Lr29* show resistance to this race (Nayar et al., 1987). The segregation for susceptibility observed in the crosses of the mutant with the above seven near-isogenic wheat lines suggests that the changes identified here are not a result of out-crossing with any of the genes, and that the new variability appears to have been generated following mutagenic treatment. The high degree of resistance in mutants of a widely adapted high yielding variety WH 147 against these races may prove useful as a new source of resistance in breeding programmes.

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Short communication

EFFECT OF TRANSFERRED RUST RESISTANCE GENES ON YIELD PERFORMANCE IN HEXAPLOID WHEAT

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Isogenic lines carrying various rust resistance genes (*Lr19, Lr24, Lr26, Lr28, Sr25, Sr26, Sr27, Sr31, Yr9*) in two Indian wheat backgrounds (HD 2009, HD 2380) were evaluated for grain yield performance under rust-free conditions. Constituted lines carrying the leaf rust resistance gene *Lr28* or the stem rust resistance gene Sr27 gave significantly lower yields when compared to the control chemically treated with Tilt 0.5%. Constituted lines carrying rust resistance genes from rye addition line 1R gave a higher yield than the untreated control, but the yield was comparatively lower when compared to the treated control. Lines carrying the rust resistance gene complex *Sr31+Lr26+Yr9* from Veery 'S' gave the highest yield in the genetic backgrounds of both the Indian wheat cultivars.

Key words: rust resistance genes, yield performance, wheat

Introduction

The transfer of alien genes to wheat generally results in a variation in the yield of the recurrent parent. The yield will be either increased or decreased, depending upon whether the transferred gene is associated with any linkage drag or not. The derivatives carrying leaf rust resistance gene *Lr19* from *Agropyron elongatum* could not be used because of the presence of linked genes for inferior baking quality (Knott, 1978). The et al. (1988) compared the effect of eight sources of stern rust resistance on the grain yield in nine Australian wheats. They observed a significant yield depression in some of the resistant lines as compared to the susceptible ones. The present communication reports the effect of several transferred rust resistance genes on grain yield in the genetic backgrounds of two Indian wheat cultivars.

Materials and methods

Four rust resistance genes (*Lr19, Lr24, Lr26, Lr28*), four stem resistance genes (*Sr25, Sr26, Sr27, Sr31*) and one stripe rust resistance gene (*Yr9*), present either individually or in combination in different hexaploid wheat stocks or alien addition lines, were transferred into two Indian wheat cultivars, HD 2009 and HD 2380. Near-isogenic rust resistance lines were constituted in BC_2F_5 and BC_4F_5. The grain yields of the selected lines were compared with those of the respective recurrent parents under rust-free conditions. Each of the constituted lines was grown in a 3 × 2 metre plot and replicated four times. The original (recurrent) parents and the constituted lines were treated with Tilt (0.5%). The first application of the chemical was made at the time of

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the initial appearance of the rust, followed by three other applications at intervals of 15 days. In this trial, absolute checks (without chemical spray) were maintained and were sprayed with water. Finally the plot grain yield was recorded, expressed in quintals/hectare, and the differences were expressed as a percentage.

Results and discussion

The grain yields of the constituted lines (BC_2F_5 and BC_4F_5) were compared with the respective recurrent wheat parents under rust-free conditions. The data on the percentage grain yield differences of the parents and the constituted lines are presented in Table 1. The data show that the yield was increased in all the constituted lines except those carrying *Lr28* in HD 2009 and *Sr27* in HD 2380. The increase was about 33–53% over the untreated check, and 0.35%–8% over the treated check. When compared to the chemical-treated control, a significant yield depression (1.2 to 34%) was noticed in constituted lines that derived from crosses HD 2009 × CS 2D/2M 3/8 (*Lr28*), HD 2380 × W 3353 (*Sr27*), HD 2009 × 1R (*Sr31+Lr26+Yr9*) and HD 2380 × 1R, while in all other constituted lines, the grain yield was significantly higher (0.35 to 53%) than those of the respective recurrent parents. Among the various rust resistance genes, lines carrying rust resistance gene complex *Sr31+Lr26+Yr9* from Veery 'S' gave the highest yields in the genetic backgrounds of both the Indian wheat cultivars.

There are a few earlier reports of yield variation associated with the transfer of rust resistance gene(s). Based on a study of the effect of the 1BL/1RS translocation on yield and yield components in 131 F_3-derived hexaploid wheat lines, Morena-Sevilla et al. (1995) concluded that the expression of 1RS depended upon the genetic background in which it was placed. Islam and Shepherd (1991) reported that a significant yield depression occurred in rust-resistant lines with *Sr26* and *Sr21* compared to susceptible lines, giving 9 and 7% lower yields, respectively. A gene responsible for crown rust resistance in oats gave a higher yield when it was derived from *Avena sterilis,* while the same gene gave a lower yield when it was derived from *A. strigosa* (Brinkman and Frey, 1977). On the other hand Dyck (1992) did not observe any deleterious effect of stem rust resistance gene *Sr40* when transferred from *Triticum araraticum* to bread wheat. Similarly, four of the eleven rust-resistant lines developed by Knott and Dvorak (1981), and 10 of the 11 rust-resistant lines developed by Zeven et al. (1983) exhibited superior agronomic performance, including grain yield. Drijepondt et al. (1990) observed that the Thatcher backcross derivative RL 6058, carrying the leaf rust resistance gene *Lr34,* out-yielded the parent by 0.3%. Considering the absence of any yield-depressive effects of a leaf rust resistance gene in wheat variety Agent (Knott, 1989) or of stem rust resistance genes in wheat cultivar LMPG (Knott, 1993), the inconsistency (low or high) of the effect of the same gene on yield in different years (Knott, 1993) and locations (Knott, 1989, 1993), and the variation in yield either due to various rust resistance genes in a particular wheat genetic background (Knott, 1993) or to different rust resistance sources (Knott, 1989), it is evident that the effect of resistance gene(s) on yield varies with different genotypic backgrounds, different genes, different sources of rust resistance and the environment.

Table 1
Comparative mean grain yield (quintal/hect.) and percentage grain yield difference of the Indian wheat parents and the constituted lines

No.	Parents/Constituted line	Generation	Mean grain yield/(Q/ha)	Grain yield difference (%)	
				Original	Treated
1	HD 2009	BC_2F_5	27.65	–	–30.26
		BC_4F_5	27.65	–	–30.26
2	HD 2009	BC_2F_5	39.65*	+43.40	–
	(Chemically treated)	BC_4F_5	39.65*	+43.40	–
3	HD 2009 Veery 'S'	BC_2F_5	42.29*	+52.95	+6.66
	(*Sr31+Lr26+Yr9*)	BC_4F_5	41.85*	+51.36	+5.55
4	HD 2009/Darf/3Ag/Kite	BC_2F_5	40.86*	+47.78	+3.05
	(*Sr26+Lr24*)	BC_4F_5	40.06*	+44.88	+1.03
5	HD 2009/W 3353	BC_2F_5	39.91*	+44.34	+0.66
	(*Sr27*)	BC_4F_5	39.87*	+44.19	+0.55
6	HD 2009/CS 2D/2M 3/8	BC_2F_5	26.31	–4.85	–33.64
	(*Lr28*)	BC_4F_5	26.15	–5.42	-34.05
7	HD 2009/1R	BC_2F_5	38.53*	+39.35	–2.82
	(*Sr31+Lr26+Yr9*)	BC_4F_5	37.05*	+34.00	–6.56
8	HD 2009/Agrus/7*Thatcher	BC_2F_5	39.85*	+44.12	+0.50
	(*Lr19+Sr25*)	BC_4F_5	39.75*	+43.90	+0.35
	CD (P=0.05%)	BC_2F_5	1.12	–	–
		BC_4F_5	1.32	–	–
1	HD 2380	BC_2F_5	29.80	–	–25.31
		BC_4F_5	29.70	–	–25.49
2	HD 2380	BC_2F_5	39.90*	+33.89	–
	(Chemically treated)	BC_4F_5	39.85*	+34.21	–
3	HD 2380/Veery 'S'	BC_2F_5	43.18*	+44.89	+8.22
	(*Sr31+Lr26+Yr9*)	BC_4F_5	42.89*	+44.41	+7.60
4	HD 2380/Darf/3Ag/Kite	BC_2F_5	42.96*	+44.16	+7.67
	(*Sr26+Lr24*)	BC_4F_5	41.43*	+39.49	+3.94
5	HD 2380/W 3353	BC_2F_5	28.64	–3.88	–28.22
	(*Sr27*)	BC_4F_5	28.35	–4.54	–28.88
6	HD 2380/CS 2D/2M 3/8	BC_2F_5	40.20*	+34.89	+0.75
	(*Lr28*)	BC_4F_5	40.00*	+34.68	+0.35
7	HD 2380/1R	BC_2F_5	39.42*	+32.28	–1.20
	(*Sr31+Lr26+Yr9*)	BC_4F_5	39.20*	+31.98	–1.65
8	HD2380/Agrus/7*Thatcher	BC_2F_5	40.64*	+36.37	+1.85
	(*Lr19+Sr25*)	BC_4F_5	40.47*	+36.26	+1.53
	CD (P=0.05%)	BC_2F_5	1.09	–	–
		BC_4F_5	1.31	–	–

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Review

WHEAT POWDERY MILDEW RESISTANCE GENES AND THEIR APPLICATION IN PRACTICE

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The wheat powdery mildew disease is the result of the interaction between wheat (host plant) and *Erysiphe graminis* DC. *f. sp. tritici* Marchal (biotrophic parasitic fungus). On some occasions, in epidemic years, considerable damage is caused, making it essential to control the pathogen. Biological plant protection based on the development and cultivation of resistant varieties could be an effective method, involving the least danger to the environment and human health. This method requires a knowledge of resistance genes, the varieties bearing these genes, the efficiency of the genes and their territorial distribution.

According to the data, the majority of cultivated varieties contain no known major resistance genes, while some varieties contain one, two or more resistance genes. Gene combinations (or gene pyramids) are considered to be effective in overcoming the pathogen. To date 24 major resistance genes have been identified and located on the chromosomes. Of these nine have been found alone or in combination in cultivated varieties. These genes are much the same in most countries, which means that the genetic diversity of powdery mildew resistance is very small. These genes no longer provide satisfactory protection, as confirmed by the frequency with which the pathogen is found to be virulent to them. As a result of their ineffectiveness, severe epidemics could develop over considerable areas.

A knowledge of the genetic background of the host plant is important in elaborating a resistance breeding strategy, in utilising resistance genes in breeding and cultivation, and in tracing changes in the race composition and virulence of the pathogen population.

Key words: wheat, wheat powdery mildew, host plant–pathogen relationship, powdery mildew (Pm) resistance genes

Introduction

The key to profitable production is to economise with natural resources. This can be promoted by the rational use of abiotic and biotic resistance in production. Plant protection, environmental protection and health safety are closely connected and have their biological basis in the development and cultivation of resistant varieties. For this reason, studies on the relationship between the host plant and the pathogen are gaining more and more ground.

A knowledge of the interactions between the genetic systems of the host plant and the pathogen is an important factor in breeding programmes aimed at the development of resistant varieties. The resistance breeding strategy must be based on the natural relationships within the pathosystem. This requires a knowledge of the resistance genes and their efficiency.

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The present paper reviews major literary sources dealing with the resistance genes responsible for resistance to wheat powdery mildew, including information on resistance genes, their location on the chromosomes, their effect on the pathogen and a list of varieties bearing resistance genes.

Relationship between the host plant and the pathogen

The systems existing in nature were recognised by mankind at a relatively early date. Biology investigates the complex system of living processes, which consists of a number of subsystems. Velich (1982) regarded the ecosystem as the operational unit of the biosphere. Zadoks and Schein (1979) made a distinction between agro-ecosystems created through human activities, the farming system, cropping regimens and the pathosystem.

Complex systems are also involved in plant protection, due to the interrelationship between the host plant and the pathogen. The gene centre theory set up by Vavilov (1935) was further developed by Zhukovskii (1971), who considered that the plant and its pathogens evolved from the same location, thus involving a process of co-evolution. In the gene centres not only the plants, but also their pathogens exhibit a wide variety of forms. Thus, in the common homeland new races of pathogens evolve together with the new plant species. Leppik (1970) enlarged this theory, suggesting that resistance gene centres developed in places where the plants were exposed to selection pressure by the pathogen for the longest period, since it was here that the pathogenicity of the pathogen achieved the greatest variability. It follows from this that the evolution of the parasite closely follows that of the host plant, and vice versa. This concept was supported genetically by the "gene for gene" theory of Flor (1956).

Based on the genetics and evolution of the interaction between the host plant and the pathogen populations, Robinson (1976) elaborated the pathosystem theory, an important distinctive feature of which is parasitism in the broad sense. The components of the pathosystem, regarded as a subsystem of the ecosystem, are the pathodemes (members of the host plant population with various degrees of resistance) and the pathotypes (members of the pathogen population with various degrees of pathogenicity). Using the nomenclature applied by breeders, the pathodeme is the variety and the pathotype the physiological race.

Since plants are generally resistant to the majority of pathogens, freedom from disease is the normal state, not susceptibility and infection (Van der Plank, 1963). For this reason, if the plant is attacked by a pathogen, it responds with a defensive mechanism. Under the given growing (agroecological) conditions numerous transitions can be observed between the two extremes of the host plant–pathogen relationship: incompatibility (resistance) and compatibility (susceptibility), leading to plants being moderately resistant, moderately susceptible, etc. The host–parasite relationship is determined on the one hand by the resistance or susceptibility of the host plant, and on the other by the pathogenicity (virulence or avirulence) of the pathogen (Fig. 1). These traits are generally genetically determined. The intensity of infection depends on the simultaneous presence or absence of these factors.

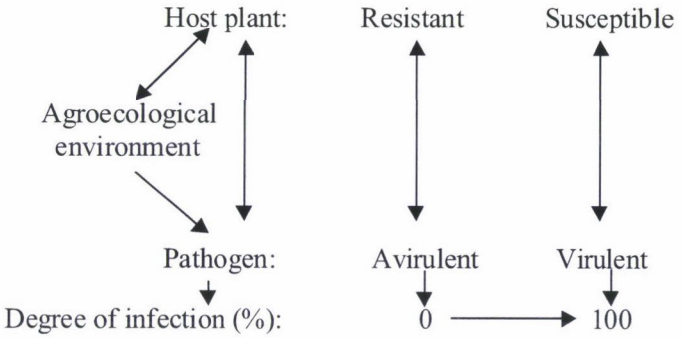

Fig.1. Relationship between the environment, the host plant and the pathogen

Due to the spread of the pathogens, substantial losses may be incurred in epidemic years, particularly if susceptible varieties are grown. These losses can be reduced by preventing the host plant and the pathogen from reacting with each other, or by making this contact more difficult. The best way of doing this is to exploit the possibilities latent in integrated plant protection. This means that the most suitable plant protection agent for the control of the parasitic microorganisms is selected from the wide range of chemicals available and is incorporated into the production technology, together with the agronomic measures required. It also involves the cultivation of resistant varieties.

A large number of authors have dealt with the genetics of resistance. In addition to traditional techniques, more and more papers are being published on the clarification of the action mechanisms of resistance genes using biotechnological methods. A detailed review of these papers was published by Király and Hornok (1996).

Two main types of plant resistance are recognised: inherited and acquired. The present review discusses inherited resistance in the case of wheat and wheat powdery mildew. Information is presented on major resistance genes, their location on the chromosomes, their origin, a large proportion of the varieties bearing these genes, and the application of these genes to date in biological plant protection in a number of European and non-European countries.

There are various types of inherited resistance. A discussion of these is not included in the present paper, as they were not the subject of the investigations. The authors are in agreement with Fodor et al. (1998), who stated: "Inherited resistance could be used in practice in an inestimable number of ways, but there are also specific limits to its application".

Genes responsible for resistance

Powdery mildew is one of the most widespread diseases of wheat. The pathogen responsible for the disease is the heterothallic, biotrophic, parasitic fungus *Erysiphe graminis* DC. *f. sp. tritici* Marchal, which belongs to the family

of powdery mildew fungi *(Erysiphaceae)* in the *Erysiphales* order of the *Ascomycetes* class. It has recently been renamed *Blumeria graminis* (DC.) Golovin.

The development of an epidemic is influenced fundamentally by three factors (Szepessy, 1977): 1. the properties of the pathogen, 2. the properties of the host plant, and 3. the environmental conditions. In recent years human activities have been mentioned as a fourth factor (Zadoks and Schein, 1979). Among these factors, the genes responsible for powdery mildew resistance and the most important varieties bearing these genes are listed in Tables 1 and 2. As far as possible these tables were compiled with reference to reviews, thus reducing the number of publications cited in the list of references. The detailed description of the first four resistance genes (*Pm1–Pm4*) was given by Briggle (1969). Later publications by Bennett (1984), McIntosh et al. (1993) and Hanusova (1995) provided valuable data on 9, 19 and 20 *Pm* genes, respectively. Information on genes *Pm21*, *Pm22*, *Pm23* and *Pm24* is to be found in papers by Chen et al. (1995), Peusha et al. (1996b) and Huang et al. (1997a). The d, e and f alleles of the *Pm3* gene were described by Zeller et al. (1993b).

Originally the wheat powdery mildew resistance genes were designated by Ml with a letter in subscript (Moseman, 1966). At the suggestion of Briggle (1969) this was changed to *Pm* (for powdery mildew) plus a number. So far 24 resistance genes have been identified and located on the chromosomes. *Pm3* has six known alleles and *Pm4* two. The genes may exert their effects independently or in various combinations. Resistance genes have been identified in a large number of varieties. Genes resistant to the pathogen could be effective tools in biological plant protection.

Based on the variety Chancellor, Briggle (1969) developed 12 near-isogenic lines using the resistance genes *Pm1*, *Pm2*, *Pm3* and *Pm4* and the variety Michigan Amber. This accelerated research on the genetic differentiation of the pathogen, leading to the development of isogenic lines of Hope (Johnson et al., 1979) and Amigo (Lowry et al., 1984).

As genetic research into powdery mildew resistance has a relatively short history, contradictions are sometimes to be found in the literature. The resistance gene *Pm4* (=*Pm4a*), for instance, was first identified in the wheat varieties Khapli and Yuma (Briggle, 1966). Isogenic lines were then developed by crossing with Chancellor. According to investigations carried out by Moseman et al. (1980) Khapli contained not one but three resistance genes, while Yuma contained two, and the isogenic lines only one. All of them, however, contained *Pm4a*. The resistance gene found in Avrora is *Pm8*, located on chromosome 1B (McIntosh et al., 1993). According to data published by Ganeva and Bochev (1976) two genes, located on chromosomes 1A and 3B are responsible for the resistance of Avrora. The situation is even more complicated for one of the lines in the differentiation series, Halle st. 13471. It is generally thought that the resistance of this line is controlled by the genes *Pm2+Mld* (Bennett, 1984). Meyer (1977), however, is of the opinion that the gene designated in this line as *Pm2* is really *Pm7*, located on chromosome 1B, while the *Mld* gene found on

Table 1
Wheat powdery mildew resistance genes and genotypes bearing the genes

Resistance gene		Chromosome	Origin of resistance	Genotype
Pm	Synonym			
Pm 1	*Mlt, Mla*	7 AL	*T. aestivum*	Anfield, As II, Axminster, Birdproof, Bonus, Converse, CI. 13836, Fedka, Festival, Huron, Kenora, Norka, Pika, Sweden, W1230, Thew, Tu 4
Pm 2	*Mlu, Mlx*	5 DS	*Ae. squarrosa*	Agent, Austerlitz, Avalon, Berlioz, Bounty, Bussard, CA 9238, Fenman, Galahad, GZC-130-39-2, Gorbi, Linyuan 7069, Longbow, Lontoi, Maris Beacon, Maris Nimrod, Maris Templar, Nandu, Norman, Obelisk, Orestis, Red Fern, Ulka
Pm 3a	*Mla*	1 AS	*T. aestivum*	Asosan, Coker 797, Florida 301, Florida 302, Hadden, PI 46890, Saluda, TA 379, Tylor
Pm 3b	*Mlc*	1 AS	*T. aestivum*	Chul
Pm 3c	*Mls*	1 AS	*T. aestivum*	Borenos, Cownpore, Hindukush, Indian, Sonora, Sturgeon
Pm 3d	*Mlk*	1 A	*T. aestivum*	Herold, Hjan Tapio, Kolibri, Munk, Ralle, Sokrates, Star, Syros
Pm 3e		1 A	*T. aestivum*	W 150
Pm 3f		1 A	*T. aestivum*	Michigan Amber × Chancellor
Pm 4a	*Pm 4*	2 AL	*T. dicoccum*	Khapli, Valgerado, Xiajian 5, Yuma, Yunyin 2, Zhengzhou 831
Pm 4b	*Mle*	2 AL	*T. carthlicum*	Achill, Agronom, Ajax, Aladin, Armada, Aron, Boheme, Botri, BPM 16, BPM 17, Combi, Fakon, Fakta, Faktor, Famos, Fazit, Gerbier, GH 67, Hana, Hermes, Horizont, K15560, M-022-92, Manu, Maris Halberd, Max, Olymp, Orbis, Pernel, Renan, Roazon, Ronos, Sabina, T 186, TP 229, Tristan, VPM 1, Weihenstephan M1
Pm 5	*Mli, mlH*	7 BL	*T. dicoccum*	Alidos, Aotea, Aquila, Caldwell, Carimulti, Carıplus, Chaoyuan 4, Dolomıt, Falke, Flanders, Forno, GA 1123, Glenwari. H-44, Hardired, Hongtuton, Hope, Ibis, Ilona, Kontrast, Kormoran, Kraka, Kutulskaya, Mailidıuo, Mercia, Milan, Nadadores 63, Purdue 5517, Redcoat, Regina, Reiher, Rektor, Selpek, Severin, Sicco, Siete Cerros, Songhuajiang 2, Sperber, Tarasaque, Tukan, Urban, Warigo, Wattines, Zhengzi 8748, Zolotistaya
Pm 6	*Mlf*	2 B	*T. timopheevii*	Abe, Claudius, Coker 747, IVGS line C, Longmai 8, Mengavi, Oasis, Omskaya 23, Omskaya 24, Thesée, Timgalen, TP114/2 × Starke deriv B
Pm 7		4A-2R, 4BS	*S. cereale* (Rosen)	Fedsec, Transec, Transfed

Table 1 (cont.)
Wheat powdery mildew resistance genes and genotypes bearing the genes

Resistance gene		Chromosome	Origin of resistance	Genotype
Pm	Synonym			
Pm 8		1B/1R, 1BL/1RS	*S. cereale* (Petkus)	Almus, Aiyuandong 3, Ambassador, Avrora, Benno, Bezostaya 2, Burgas 2, Clement, Disponent, Fenkang 2, Gk Bence, Gk Ságvári, Gk Szemes, Gk Tiborc, Götz, Halle stamm 1444, Huapei 28, Ji 5418, Jindong 1, Jing 411, Kavkaz, Livia, Lovrin 10, Lovrin 13, Lumai 5, Marina, Miangyang 8620, Mildress. Mironovskaya 10, Mv 15, Neuzucht, Niklas, Odilo, Omskaya 25, Orlando, Predgornaya, Riebesel 4751, Roseana, Saladin, Salzmünder Bartweisen, Samanta, Senta, St 1444, Stuart, Skorospelka 35, Tambo, Voyage, Veery, Weique, Winnetou, Wulian 3, Yan 1604, Zorba
Pm 9		7 AL	*T. aestivum*	Not yet isolated alone, only linked to other resistance genes (see Table 2)
Pm 10		1 D	*T. aestivum/ T. spelta duhamelianum*	Norin 4, Norin 26, Norin 29, Penjamo 62, Shin- chunaga
Pm 11		6 BS	*T. aestivum*	Chinese Spring, Salmon, *T. compactum* No. 44
Pm 12		6 BS-6 SS/6 SL	*Ae. speltoides*	Vembley derivative, 6 BS-6 SS/6 SL
Pm 13		3B, 3D	*T. longissimum*	*T. longissimum* derivatives, 3BL/3SS
Pm 14		6B	*T. aestivum*	Not yet isolated alone, only linked to other resistance genes (see Table 2)
Pm 15		7 DS	*T. aestivum*	Not yet isolated alone, only linked to other resistance genes (see Table 2)
Pm 16		4A	*T. dicoccoides*	*T. dicoccoides* derivatives, BRG 3N
Pm 17		1AL-1RS	*S. cereale,* (Insave)	Amigo
Pm 18		7A	*T. aestivum*	Weihenstephan M1N
Pm 19		7D	*Ae. squarrosa*	Synthetic xx 186
Pm 20		6BS-6RL	*S. cereale* (Prolific)	KSWCRC 27, KS 93 WGRC 28,
Pm 21		6AL-6VS	*Haynaldia villosa*	92-1009
Pm 22		1D	*T.aestivum*	Elia, Est Mottin, Ovest, Tudest, Virest
Pm 23			*T. aestivum*	81-7241
Pm 24		6D	*T. aestivum*	Chiyacao
	Mld	4B	*T. durum*	Not yet isolated alone, only linked to other resistance genes (see Table 2)

Table 2
Wheat powdery mildew resistance gene combinations and the genotypes bearing them

Gene combination	Genotype
Pm 1 Pm 3a	BGRC 44514
Pm 1 Pm 4b	Rang, K 4l40-1
Pm 1 Pm 9	Anfield, Pompe, Ring
Pm 1 MlGa	Galizischer Grannen, Heines Japhet, Oberfläzer Landweisen
Pm 1 Pm 2 Pm 4b	Attis, Solo, Troll
Pm 1 Pm 2 Pm 6	Omskaya 28
Pm 1 Pm 2 Pm 9	Mephisto, Normandie
Pm 1 Pm 4b Pm 9	Remus
Pm 1 Pm 5 Pm 8	7198/93, 714793
Pm 1 Pm 2 Pm 4b Pm 9	Planet, Sappo, Sunnan
Pm 1 Pm 2 Pm 4b Pm 6 Pm 9	Nemares, Walter
Pm 2 Pm 3d	Axona, 93-6009
Pm 2 Pm 4a	93-6330, 92-1014, 92-1015
Pm 2 Pm 4b	Compal, Simona, Timmo
Pm 2 Pm 5	Gk Örzse, Pagode, Tambor
Pm 2 Pm 6	Agra, Adular, Asta, Avir, Brigand, Brimstone, Champtal, CI 12632, CI 12633, Erliyan 94-1-2, Estica, Friedland, Gawain, Heiduck, Hustler, Kharkhovskaya 12, Kinsman, Konsul, Lutescens 137/87-13, Mardler, Maris Fundin, Maris Huntsman, Mv 11, Mv 13, Nougat, Omskaya 18, Ortler, Somara, Sana, Torysa, TP 114, Virtue, Zagrepchanka
Pm 2 Pm 8	Damier, Hornet
Pm 2 Pm 9	L 4/1, L 12/2
Pm 2 Mld	Halle stamm 13471, H / 8810/47, Maris Dove,
Pm 2 Pm 4b Pm 6	Andros, Contra, Guinong 87-39, Lonja, Rendezvous, Timmo, Walter
Pm 2 Pm 4b Pm 8	Apolló, Kontra, Sofia, Sparta
Pm 2 Pm 5 Pm 6	Crossbow, Parade
Pm 2 Pm 5 Pm 8	Mv 17
Pm 2 Pm 6 Pm 8	CWW 1645-5, Euris, Ritmo, Xanthos
Pm 2 Pm 4b Pm 5 Pm 6	Bovictus
Pm 2 Pm 4b Pm 6 Pm 8	Knirps
Pm 3d Pm 4b	Kadet, Turbo
Pm 4a Pm 8	Kenquia 1, Xiaoheixiao, Zhengzi R 85100
Pm 4b Pm 5	Avir, Boxer, Clan, Dijana, Divo, Devon, Fakir, Ibis, Irena, Kadett, Kiwi, Kontrast, Mission, Zdar
Pm 4b Pm 6	Bai Mian, Solveig, Sorbas, 93-6317, 93-6318
Pm 4b Pm 8	Branka, Ganfeng 2, Glockner, Herzog, Kronjuwel, Roxana, Shannong 830076, Selacta, Sida, Qianhua 4, Toronto, Toroub, Weihenstephan 623/65
Pm 4b MlAr	Reno, Runan
Pm 4b Pm 5 Pm 8	Mv 21-86
Pm 4b Pm 6 Pm 8	Brigadier
Pm 5 Pm 6	Adriana, Arthur, Bert, Coker 983, Double Crop, Greif, GK Mini Manó, Lambros
Pm 5 Pm 8	Danubia, Granada, GK Zombor, Iris, Kristall, Mv 14, Mv 16, Mv 18, Sensor, 4304/93
Pm 6 Pm 8	Albrecht, 4044/93
Pm 10 Pm 11	T. spelta duhamelianum
Pm 10 Pm 15	Norin 4, Norin 26, Shin-chunaga, T. macha subletschumicum
Pm 11 Pm 15	Chinese Spring, T. compactum No. 44
Pm 14 Pm 15	Akabozu, Kokeshikomugi, Norin 10

chromosome 5D is in fact *Pm2b*, since it is an allele of the gene *Pm2*, which he thus suggests should be renamed *Pm2a*. According to Bennett (1984) the varieties Arthur, Arthur 71, Abe and Oasis all contain the resistance genes *Pm2* + *Pm6*, while Leath (1991) and McIntosh et al. (1993) report the resistance of Abe and Oasis to be due to *Pm6* and that of Arthur to be attributable to *Pm5* and *Pm6*. According to Leath (1991), Arthur 71 contains no resistance genes.

Even greater discrepancies may be found when a variety contains several resistance genes. The variety Ritmo, for example, contains resistance genes *Pm2*, *Pm5* and *Pm6*, according to Hanisová et al. (1997), or resistance genes *Pm2*, *Pm4b*, *Pm6* and *Pm8*, according to Ecker and Lein (1994). Three different gene formulae have been published for the variety Walter: *Pm2*, *Pm4b*, *Pm6* (McIntosh et al., 1993), *Pm1*, *Pm2*, *Pm4b*, *Pm9* (Winzeler et al., 1991) and *Pm1*, *Pm2*, *Pm4b*, *Pm6*, *Pm9* (Hartl et al., 1996). The above-listed data are quite sufficient to confirm the statement of Babayants et al. (1985) who argued that the manifestation of powdery mildew resistance is much more complex than may be thought on the basis of the experimental data available. Nevertheless, despite the slight discrepancies, these gene formulae provide valuable guidelines as to the nature of the genes identified in different varieties.

The origin of the genes

Some of the genes listed in Table 1 were identified in common wheat, while others were introduced into *T. aestivum* by means of distant hybridisation. A few are only to be found in wild species. The varieties bearing resistance genes listed here originate from various parts of the world. In addition to Hungarian varieties, those to be found in neighbouring countries were also collected, together with those grown in regions with similar agroecological conditions and genotypes of exotic origin. Breeders are thus able to include sources of different agronomic value, containing different resistance genes in their breeding programmes.

Below, the resistance genes will be grouped and discussed according to the origin of the resistance.

Eincorn (diploid) series

Powdery mildew resistance genes were isolated from three species previously classified as *Aegilops*.

Triticum longissimum (Schweinf-Muschli) Bowden = *Aegilops longissima* S. (*Pm13*)

The *Pm13* powdery mildew resistance gene originates from this species (McIntosh et al., 1993). It has not yet been transferred to cultivated wheat varieties.

Triticum speltoides (Tausch) Gren ex Ricter = *Aegilops speltoides* Tausch (*Pm12*)

The *Pm12* resistance gene was isolated in the species *Aegilops speltoides*. Breeders have not yet exploited the possibilities latent in this gene.

Triticum tauschii (Coss.) Schmal. = *Aegilops squarrosa* L. (*Pm2, Pm19*)

The powdery mildew resistance of *Aegilops squarrosa* L. is excellent. The D genome of common (hexaploid) wheat originates from this species (Barabás, 1987), which is why, according to Bennett (1984), the transfer of its resistance into cultivated wheat varieties did not cause any great difficulty. Two genes have been identified from this species to date (McIntosh et al., 1993), of which *Pm19* is the result of a synthetic hybridisation, while *Pm2* is carried by numerous genotypes and cultivated varieties. In contrast to *Ae. squarrosa*, these wheat varieties become heavily infected with powdery mildew under Hungarian conditions. This can be attributed to the fact that 90% of the pathotypes in the pathogen population are now virulent to this gene. In 1980 this figure was only 20% (Szunics and Szunics, 1995).

Emmer (tetraploid) series

Genes responsible for powdery mildew resistance have been isolated in five species of this series.

Triticum timopheevii Zhuk. (*Pm6*)

In the words of Vavilov (1966) *T. timopheevii* is a "powerhouse of immunity". Among other things, it is resistant to powdery mildew. The resistance gene *Pm6* was isolated from the 2B chromosome of this species. A large number of resistance sources have been developed using *T. timopheevii*, with the help of which a number of cultivated varieties have been bred, including Maris Huntsman, Maris Fundin, Kinsman, Virtue, Arthur, Arthur 71, etc. Their value in biological plant protection has led to them being sown on considerable areas in the UK and the USA (Bennett, 1984). They have also been used as crossing partners in the Martonvásár institute; Arthur, for instance, is to be found in the genealogy of the wheat varieties Martonvásári 10, Martonvásári 14, Martonvásári 16 and Martonvásári 18. It is also one of the parents of the varieties GK Kincső, GK Bence and GK Csűrös, bred in the Cereal Research Institute in Szeged.

In 1973 and 1974 the ears, and to an even greater extent the awn of *T. timopheevii* became heavily infected with powdery mildew, while the leaves and stem remained symptom-free. Eighteen isolates were prepared in the winter of 1974/75 from pathogen samples collected from the ears in summer 1974. The following six races were identified from these cultures with the given frequencies: 4: 50.5%, 26: 26.8%, 2, 9, 15 and 32: each 5.8%. This was not

surprising, as all these races had previously been identified from cultivated wheat varieties. According to 1974/75 data the frequency of these races in the powdery mildew population was: 4: 18.9%, 26: 25.6%, 2: 8.5%, 9: 6.1%, 15: 1.2% and 32: 10.4%. Four of these (26, 4, 32 and 2) were prevalent in the year in question. The races identified were multiplied in the greenhouse and used to inoculate seedlings emerging from the seeds of ears from which the powdery mildew was isolated. Seedlings in the 2–3-leaf stage did not become infected. They showed no signs of powdery mildew, though mild chlorotic symptoms were observed. This was no doubt a case of the organotropic specialisation of the pathogen (Szunics, 1988).

Triticum dicoccoides Körn. (*Pm16*)

The resistance gene *Pm16*, located on chromosome 4A, originates from this species (McIntosh et al., 1993). This has not been transferred to cultivated wheat varieties to date.

Triticum dicoccum Schrank (*Pm4a*, *Pm5*)

Literary data suggest that in this species resistant types are in the majority (Dorofeyev, 1976; Krivchenko et al., 1979; Moseman et al., 1984). Resistance gene *Pm4a* was identified on the 2AL chromosome of *T. dicoccum* (The et al., 1979) and *Pm5* on chromosome 7BL (McIntosh, 1983). Both have been transferred into a number of wheat varieties. Genotypes carrying gene *Pm5* are fairly frequent (Table 1), though their powdery mildew resistance is poor under Hungarian conditions.

The resistance gene *Pm4a* (=*Pm4*) was first identified in the wheat varieties Khapli and Yuma (Briggle, 1966). Isogenic lines were developed by backcrossing a number of times to Chancellor. According to studies made by Moseman et al. (1980) Khapli possesses three resistance genes, Yuma two and the isogenic lines one each, but they all contain *Pm4a*. Data obtained in Martonvásár confirm this. In field and greenhouse (race and virulence analysis) experiments carried out between 1983 and 1998 Khapli proved to be resistant in every case (having a score of 0, or occasionally 1), while the isogenic line Khapli × Chancellor was generally infected to a considerable extent (4-9), depending on the intensity of the powdery mildew attack. This was also true of Yuma × Chancellor (4-8). In experiments carried out by Winzeler et al. (1991) powdery mildew was virulent to both Yuma and Khapli × Chancellor.

Triticum durum Desf. (*Mld*)

The varieties of the species *T. durum* are generally regarded as resistant (Dorofeyev, 1976), although Zhukovsky (1971) considers this statement to be exaggerated. The resistance gene *Mld* is located on the 4B chromosome of this species (Wolfe, 1967) and is present in durum wheat varieties and advanced lines bred in Italy (Cariello et al., 1977). In recent years the resistance of a large number of durum varieties and lines has been evaluated. The majority proved to be moderately susceptible or susceptible. Very few less susceptible lines were found, and hardly any which were resistant.

Triticum carthlicum Nevski (*Pm4b*)

The *T. carthlicum* species was distinguished by Vavilov (1935) from common wheat as long ago as 1918 due to its resistance to powdery mildew. It was also found to be resistant in experiments carried out in Martonvásár. Similar observations were published by Lelley and Rajháthy (1955), though susceptible forms also exist: among 46 lots examined by Krivchenko et al. (1979) 69.5% gave scores of 0, 2.2% 1-2 and 28.3% 3-4. The resistance gene *Pm4b* (2AL) originates from this species and is responsible for the resistance of a number of varieties (The et al., 1979).

Spelt (hexaploid) series

Research results have been achieved in this series in the case of common wheat.

Triticum aestivum L. em Thell. (*Pm1*, *Pm3a*, *Pm3b*, *Pm3c*, *Pm3d*, *Pm3e*, *Pm3f*, *Pm9*, *Pm10*, *Pm11*, *Pm14*, *Pm15*, *Pm18*, *Pm22*, *Pm23*, *Pm24*)

As far as resistance is concerned the species of the *Triticum* genus differ substantially from each other, with the largest number of resistant genotypes being found among the diploids and the largest number of susceptible species belonging to the hexaploid series (Vavilov, 1935; Lelley and Rajháthy, 1955). These divisions are gradually fading away, as more and more wheat varieties belonging to the *aestivum* species have excellent resistance. Breeders have developed new resistant varieties of common wheat (Koltay and Balla, 1982), but these have little genetic diversity, as the same gene is responsible for the resistance of a great number of varieties.

It can be seen from Table 1 that 11 resistance genes (*Pm1*, the alleles of *Pm3*, *Pm9*, *Pm10*, *Pm11*, *Pm14*, *Pm15*, *Pm18*, *Pm22*, *Pm23*, *Pm24*) originate from *T. aestivum*. Some of these have been known for a long time, while others have been identified more recently. The lack of genetic potential experienced by Vavilov (1935), however, is still a problem as far as resistance to powdery mildew is concerned. This is confirmed by data published by Lebedeva (1994), who found ten times as many resistant genotypes among the diploid species and three times as many among the tetraploids as in common wheat. Over the last thirty years the ratio of pathotypes virulent to the resistance genes investigated was considerable (Szunics and Szunics, 1995). In the case of the resistance gene *Pm1*, for instance, this ratio ranged from 44 to 76%, while the range was 61 to 99% for *Pm3*. Despite the fact that these genes are not found in varieties cultivated in Hungary, the ratio of pathotypes virulent to them is high.

Special mention should be made of resistance genes *Pm10*, *Pm11*, *Pm14* and *Pm15*, which were isolated using a strain selected from hybrids originating from a cross between *E. graminis f. sp. tritici* and *E. graminis f. sp. agropyri*. These genes are not effective against wheat powdery mildew, but provide protection against the powdery mildew fungus attacking wheatgrass (Tosa, 1989).

Over the last few years the powdery mildew resistance of genotypes bearing these genes has been evaluated in the Martonvásár nursery. In general they were found to be more susceptible in every year than the susceptible control, Bezostaya 1. In Hungary these Japanese wheat varieties are extremely sensitive not only to powdery mildew, but also to cereal viruses.

Related genera

Secale cereale L. (*Pm7*, *Pm8*, *Pm17*, *Pm20*)

In the second half of the last century research into the possibility of crossing wheat and rye was aimed chiefly at satisfying scientific curiosity. The *Triticale* developed by Rimpau from such crosses held promise of practical gain, however (Kiss, 1968). In many cases wheat genotypes segregating from the wheat × rye hybrids inherited certain agronomically valuable properties from rye, such as disease resistance. Four powdery mildew resistance genes have been determined which originate from rye (*Pm7*, *Pm8*, *Pm17*, *Pm20*). Of these, only varieties bearing *Pm8* have any economic value.

The *Pm7* gene is the result of a translocation between the 2R chromosome of the rye variety Rosen and the 4A chromosome of wheat (Bennett, 1984), although McIntosh et al. (1993) consider the 4BS chromosome to be involved. This gene is to be found in a number of wheat varieties which are not in general cultivation. According to Lebedeva (1994) it only exerts its effect in adult plants. Under Hungarian conditions the productivity and lodging resistance of genotypes bearing the *Pm7* resistance gene are poor and they are susceptible to powdery mildew. Ninety percent of the races in the pathogen population are virulent to this gene.

Genotypes containing the 1BL/1RS translocation were developed independently at two research stations (Weihenstephan and Salzmünde) in Germany in the thirties using the rye variety Petkus. Later the genes responsible for their excellent powdery mildew (*Pm8*), leaf rust (*Lr26*), stem rust (*Sr31*) and yellow rust (Yr 9) resistance were identified. These wheat-rye hybrids were imported into the Soviet Union, where their biological and economic properties were evaluated at various research stations attached to VIR. These genotypes caught the attention of Lukyanenko (1966), who used them in breeding the varieties Avrora, Kavkaz, Bezostaya 2 and Predgornaya, which were of real economic value and were grown or used as basic breeding stock in various parts of the world. These varieties or the original genotypes were then included in breeding programmes almost universally, thus leading to a gene monoculture and the possibility of genetic vulnerability (Szunics, 1988). This is confirmed by the data of Villareal et al. (1991), who found that some 50% of the advanced lines in the CIMMYT wheat breeding programme in 1988 contained the 1B/1R translocation. Wheat varieties of this type were cultivated on a total of 25 million hectares worldwide in 1990.

The work underway in the Martonvásár institute in this field was summarised by Bedő et al. (1993) as follows: "The Martonvásár wheat varieties bearing the 1B/1R translocation are now in the fifth or sixth breeding cycle since the development of the original wheat-rye translocation in the variety Neuzucht, and due to the differing genetic backgrounds and selection environments they exhibit a wide range of agronomic characters. Most of the 1B/1R Martonvásár wheat varieties examined, with the exception of Mv 18 and Mv 21, have efficient resistance to stem rust due to the presence of *Sr31*. As *Lr26* and *Pm8* do not provide effective protection from the prevalent races, considerable differences could be observed in leaf rust and powdery mildew resistance. A similar conclusion was reached when testing breadmaking quality. In many varieties it proved possible to eliminate the detrimental effect of the 1R rye segment on this property, judging by the results obtained for loaf volume, farinograph index and farinograph water absorption ability. The opinion that the 1B/1R translocation necessarily leads to poor breadmaking quality has been refuted. At the same time these varieties have increased production, giving them an important role in general cultivation."

According to Szunics (1988) and Szunics et al. (1991), under Hungarian conditions the wheat varieties Avrora and Kavkaz were resistant to powdery mildew, leaf rust and stem rust when they were state registered in 1970. Due to their many good agronomic properties they quickly gained ground, being cultivated on 43.6% of the growing area in 1974. These varieties were grown not only in Hungary, but in many other countries in the world, so the "fight for existence" began between the varieties and the pathogens. The gene responsible for powdery mildew (*Pm8*) was conquered most rapidly, due to the multiplication of the virulent races 4, 26 and 52. While these races made up only 4% of the pathogen population in 1971, they had reached 45.7% by 1974. The leaf rust resistance of the genotypes was determined by gene *Lr26*. Biotypes of race 77 virulent to this gene gradually multiplied (to 43.3% in 1977), the ratio of race 74 increased (22.3%) and race 61 appeared and rapidly spread (4.2%). According to the data of Manninger (1991) this race had a ratio of over 20% in 1991. The gene *Sr31* has so far achieved satisfactory success in the struggle to maintain stem rust resistance, fending off attacks by various races over the years. Yellow rust rarely presents a problem in Hungary.

Breeders would do well to take into consideration the fact that in wheat varieties developed using the 1B/1R translocation the powdery mildew resistance coded by the *Pm8* resistance gene is not always manifested, due to the inhibitory effect of a dominant suppressor gene (Hanusova et al., 1996).

The wheat variety Amigo, bearing the 1AL/1RS translocation, was selected from a wheat × rye (cv. Insave) hybrid population. The genes *Pm17*, *Lr24* and *Sr24* are responsible for its resistance to powdery mildew, leaf rust and stem rust, respectively. In addition the variety is resistant to aphids. Hsam and Zeller (1997) suggest that allelism exists between genes *Pm8* and *Pm17*, while Bennett (1984) draws attention to the excellent adult powdery mildew resistance, but greenhouse susceptibility of Amigo. Csősz et al. (1997) also found it to be

generally resistant in field experiments. Similar results were achieved by Szunics and Szunics (1995), who found, however, that 52-91% of the powdery mildew population was virulent to Amigo plants in the early stages. This can no doubt be attributed to ontogenetic specialisation.

Resistance gene *Pm20* originates from the rye variety Prolific and was introduced into wheat with the 6BS/6RL chromosome translocation (Friebe et al., 1994). As yet its economic value is unknown.

Haynaldia villosa (L.) Schur (*Pm21*)

Literary sources mention the resistance of *Haynaldia villosa* and of hybrids developed from it (Bennett, 1984). The powdery mildew resistance gene *Pm21* was isolated as the result of the 6AL/6VS translocation (Chen et al., 1995).

Temporarily designated resistance genes

In some genotypes the location of the resistance genes has not yet been clarified, so they have been given symbolic designations, usually consisting of the letters Ml together with a reference to the variety in which resistance was first noted (Boesen et al., 1996). Zeller et al. (1993a) were the first to report the resistance of Aristide (MlAr), which was also to be found in the varieties Abo and Courtot, which had a similar genetic background. Paderina et al. (1995) found similar resistance in the genotypes Tarskaya 5 and M-026-92.

Boesen et al. (1996) provide information on the following varieties and their resistance: Tonic (*MlTo*), Broom (*MlBr*), Sicco (*MlSi2*), Sona (*MlSo*), Talent (*MlTa2*), Axona (*MlAx*), Fresco (*MlFr*), Haven (*MlHa2*), Hereward (*MlHe2*), Cornette (*MlCo3*) and Vitus (*MlVi2*). Lutz et al. (1995) reported the resistance genes *MlBr* (Bretonischer Landweizen) and *MlGa* (Arin, Densi, Erli, Garnet, Harro, Heines Koga II, Lichtis Früh, Lichti, Nos Nordgan, Opal, Peragis Grant).

Among the many publications mention should also be made of the report given by Lebedeva (1994) of powdery mildew resistance sources developed by means of distant hybridisation. The stock designated *PmTt1*, for instance, originates from *T. timopheevii. T. macha var. paleocolchicum* (*IL6*) and *T. spelta* (*PmSp*) are also valuable sources. The resistance of varieties Harkovskaya 6, Harkovskaya 8 and Botenicheskaya 2, given the symbol *PmCh*, arises from *Elytrigia glaucum*. This gene is active in all phases of ontogenesis, while the *PmG* gene present in the variety Graekum 114 only exerts its effect in adult plants. The resistance of genotypes designated *PMIL* originates from *Triticale*.

Gene pyramids (combinations)

This expression is used when several resistance genes are accumulated in a single variety in the course of breeding. It is thus hoped that the resistance will prove lasting. Some authors feel that this will be the main direction of future research, while others doubt whether this will really solve the problem (Bennett, 1984).

On the basis of the literary sources detailed in the References, Table 1 lists the resistance genes and the varieties which carry them. Table 2 includes genotypes which contain two or more resistance genes. It can be seen from the table that many powdery mildew resistance genes and combinations are not found in economically important varieties, but in some cases they have been incorporated into a number of cultivated varieties (e.g. *Pm2*, *Pm4b*, *Pm5*, *Pm6*, *Pm8* and various combinations of these). A considerable number of varieties contain two resistance genes, especially the combinations *Pm2* + *Pm6* and *Pm4b* + *Pm8*. Very few varieties have three or more resistance genes.

Limpert et al. (1994) tested 23 genotypes originating from Hungary and published the data presented in Table 3. This work was primarily of theoretical importance, as the majority of genotypes investigated are not under cultivation, so they cannot be utilised for biological plant protection purposes. The more so, because the genes detected are genetically vulnerable under Hungarian conditions. For instance, 82.38% of the pathotypes making up the wheat powdery mildew population in 1997 are virulent to *Pm2*, 70.47% to *Pm4b*, 78.57% to *Pm5*, 79.04% to *Pm6* and 98.09% to *Pm8*. In the case of the combination *Pm2* + *Pm6*, 73.33% of the pathotypes exhibited virulence.

Table 3
Resistance genes of genotypes originating from Hungary (Limpert et al.1994)

Group	Resistance gene	Genotype
1.	none	Bánkúti 1201,. GK Csanád, GK Szőke, Mv 4, Mv 8, Mv 9, Mv 12
2.	*Pm 8*	GK Bence, GK Ságvári, GK Szemes, GK Tiborc, Mv 15, Mv 14-85
3.	*Pm 2+5*	GK Őrzse
4.	*Pm 2+6*	Mv 11, Mv 13
5.	*Pm 5+6*	GK Mini Manó
6.	*Pm 5+8*	GK Zombor, Mv 14, Mv 16, Mv 18
7.	*Pm 2+5+8*	Mv 17
8.	*Pm 4b+5+8*	Mv 21-86

Occurrence of resistance genes in varieties cultivated in various countries

The majority of the data included in Table 4 stem from Europe, with a few data from Asia and North America. Most cultivated varieties possess no major resistance genes. In spite of this, epidemics and severe losses do not occur every year. Winzeler et al. (1991) attribute this to the fact that breeders make use of fairly permanent resistance which has not yet been properly identified, at least not in Switzerland. These authors draw attention to the fact that the use of varieties with known major resistance genes could mask this valuable type of resistance, which may thus be eliminated.

In presenting the distribution of resistance genes in varieties cultivated in European countries, the data of Zeller et al. (1993c) were used in the case of the UK and Denmark. Here and elsewhere the resistance genes are to be found alone

Table 4
Occurrence of resistance genes in varieties cultivated in various countries

Country	Resistance gene								
UK	*Pm 1*	*Pm 2*	*Pm 3d*	*Pm 4b*	*Pm 5*	*Pm 6*	*Pm 8*	*Mld*	
Czechoslovakia	*Pm 2*	*Pm 3d*	*Pm 4b*	*Pm 5*	*Pm 6*	*Pm 8*			
Czech Republic	*Pm 2*	*Pm 4b*	*Pm 5*	*Pm 8*					
Slovakia	*Pm 2*	*Pm 4b*	*Pm 5*	*Pm 6*	*Pm 8*				
Denmark	*Pm 1*	*Pm 2*	*Pm 3d*	*Pm 4b*	*Pm 5*	*Pm 6*	*Pm 9*		
Finland	*Pm 4b*	*Pm 5*							
France	*Pm 1*	*Pm 2*	*Pm 4b*	*Pm 5*	*Pm 6*	*Pm 8*	*MlAr*		
Croatia	*Pm 2*	*Pm 4b*	*Pm 5*	*Pm 6*	*Pm 8*				
Hungary	*Pm 2*	*Pm 4b*	*Pm 5*	*Pm 6*	*Pm 8*				
Germany	*Pm 1*	*Pm 2*	*Pm 3c*	*Pm 3d*	*Pm 4b*	*Pm 5*	*Pm 6*	*Pm 8*	*Pm 9*
Switzerland	*Pm 1*	*Pm 2*	*Pm 3d*	*Pm 4b*	*Pm 5*	*Pm 8*	*Pm 9*		
Estonia	*Pm 1*	*Pm 4b*	*Pm 5*						
Russia (Northern Caucasus)	*Pm 2*	*Pm 4b*	*Pm 6*						
Russia (Western Siberia)	*Pm 1*	*Pm 2*	*Pm 4b*	*Pm 6*	*Pm 8*	*MlAr*			
China	*Pm 2*	*Pm 3d*	*Pm 4a*	*Pm 4b*	*Pm 5*	*Pm 6*	*Pm 8*		
USA	*Pm 3a*	*Pm 5*	*Pm 6*						

or in various combinations. The largest number of resistance genes was identified in varieties cultivated in Germany (Zeller et al., 1993c). Ecker and Lein (1994) gave details on the resistance genes of many cultivated wheat varieties and found that the majority of these genes were now ineffective, while due to the nature of breeding activities, the new resistance genes are not yet to be found in the varieties used for general cultivation. Data originating from France differ in that *Pm3* and *Pm9* are not to be found in these stocks. However, a new gene has been discovered, conditionally designated as *MlAr* (Zeller et al., 1993a, c). The powdery mildew resistance gene distribution in Switzerland (Winzeler et al., 1991) is somewhat closer to that of Germany than to that of France or Denmark. The *Pm4b* gene is to be found in varieties grown in Finland. It is also probable that the variety Heta contains *Pm5*. The resistance genes *Pm3d* and *Pm6* were identified in varieties not under general cultivation (Peusha et al., 1996).

Difficulties were encountered when processing data originating from the Soviet Union, not only because few sources are available, but also because the territorial identification was ambiguous. It is clear from the paper of Anpilogova et al. (1993) that the wheat powdery mildew population in the Northern Caucasus is very heterogeneous and virulent to the majority of resistance genes. The genes *Pm2*, *Pm4b* and *Pm6* were demonstrated most frequently in cultivated varieties, but no data were provided on other genes. The title of a paper published by Peusha et al. (1995) mentions Russian varieties, but the introduction discusses the importance of powdery mildew in the Baltic States. The majority of the co-authors live and work in Germany, and the first author in Estonia, which is why the data are listed under this heading. Three genes (*Pm1*, *Pm4b*, *Pm5*) were found in cultivated varieties. In addition, genes *Pm2*, *Pm6* and

Pm9 were responsible alone or in combination for the resistance of a number of genotypes developed as the result of distant hybridisation.

From the point of view of epidemiology, Hungary, Croatia and Slovakia form a single geographical unit. The wind is easily capable of transporting infection from one country to the other. The powdery mildew resistance genes and gene combinations in the cultivated varieties are almost identical (Huszár, 1992; Lutz et al., 1992; Hanusova and Bartos, 1993; Limpert et al., 1994). Some of the varieties cultivated in Hungary possess no major resistance genes, so they become infected fairly easily and are thus susceptible to powdery mildew. Resistance genes *Pm2*, *Pm4b* and *Pm6* are not frequent and varieties bearing these genes do not occupy significant sowing areas, so they are unlikely to have played an important role in the formation or alteration of pathogen virulence. Some 90% of the pathotypes are virulent to resistance genes *Pm2* and *Pm6*. In the seventies the pathogen was avirulent to *Pm4b*, but by the mid-eighties a few virulent pathotypes were found, the proportion of which gradually increased, reaching 30% in 1990. In recent years it has become as high as 90% or more. Due to an increase in the number of varieties with the 1B/1R translocation and in their sowing area, the proportion of races virulent to the resistance gene *Pm8* has also increased, from 38% in 1971 to 100% in 1990 and 98% in 1997.

The work of Paderina et al. (1955) threw light on the presence of powdery mildew resistance genes in Asia, in the Western Siberia region of Russia. Of the 50 varieties examined, 13 became severely infected and 7 to a moderate extent. Some of the varieties possessed no resistance genes and the remainder one, two or three genes. It is interesting to note that, with the exception of *Pm5*, they possessed the same resistance genes as varieties grown in France. In China seven resistance genes are present in cultivated varieties (Xia et al., 1995; Huang et al., 1997b). With one exception (*Pm4a*) the resistance genes were the same as those observed in Czechoslovakia. *Pm21* and the resistance gene *Pm24*, identified in the Chinese landrace Chiyacao, have been transferred into new breeding stock.

According to Leath (1991) the resistance genes *Pm3a*, *Pm5* and *Pm6*, or combinations of these, are to be found in varieties cultivated in North America.

It can be seen in the data in Table 4 that the genetic diversity of the powdery mildew resistance of cultivated varieties is extremely low; in practice the same 9 powdery mildew resistance genes are to be found alone or in combination in them all. This is an important contributing factor to the development of epidemics. A number of known, or as yet unidentified, but effective resistance genes have not yet been exploited in the development of new varieties.

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Review

THE 50TH ANNIVERSARY OF THE OPENING OF THE WORLD'S FIRST PHYTOTRON

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The first phytotron in the world was opened fifty years ago in Pasadena. Since then many buildings have been constructed in which the growth conditions of experimental plants can be programmed reproducibly, thus creating the necessary conditions for exact plant research. A new field of science has evolved: phytotronics, with its own specialists, scientific societies and conferences. Books, papers and journals on phytotronics have been published and firms in various countries have made the manufacture of plant growth chambers their chief profile. The conditions available in the phytotrons have made it possible to study not only the relationship between the plant and its environment, but also such problems as the probable effects of global climate changes on plants. Phytotronics is also involved in such fields as space research. In 1972 the first phytotron in Hungary was opened in the Agricultural Research Institute of the Hungarian Academy of Sciences in Martonvásár. With its fifty plant growth units, in which the climate of any part of the earth covered with vegetation can be programmed reproducibly from –25°C to +45°C, this is still one of the largest phytotrons in the world. The research in progress in the Martonvásár phytotron covers a wide range of topics from plant genetics to breeding. The majority of the experiments are carried out on the chief crops investigated in the institute, cereals and maize.

Key words: controlled environment, gradient chamber, phytotron, phytotronics, plant growth chamber

Introduction

Plant biologists have long dreamed of being able to create the environmental conditions required for the growth and development of experimental plants, especially the temperature, illumination, relative humidity, etc., at any time of the year, independently of the external weather conditions, in a programmed and reproducible manner. Although chambers designed for the raising of plants under artificial conditions were first constructed in the earlier part of the century, it was not until the 1940s that climate techniques, illumination techniques and electronic regulation systems had reached a sufficiently advanced state for the manufacture of plant growth chambers suitable for scientific research. The modern plant research buildings constructed to house these climatic chambers are called phytotrons from the Greek words phyto (plant) and tron (house), at the suggestion of the head of the first phytotron built in Pasadena in 1949 (Went, 1950). Since then, many phytotrons have been

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built in which the plant growth conditions can be programmed reproducibly, thus making it possible to carry out exact plant research.

The scientists who specialised in this new field of research, phytotronics, set up scientific associations and organised conferences for the dissemination of information. Books, papers, journals and other publications have been published on the subject of phytotronics, while special factories have been set up to manufacture plant growth chambers.

Apart from the creation of exact plant research conditions and the simulation and reproduction of the natural environment, phytotron units have also made it possible to examine the relationship between the plant and its environment in a novel way. The effect of the environment can be broken down into its component parts and plant responses to each programmed parameter can be separately analysed.

In addition to current plant research tasks, it is also possible to study future problems such as the probable effects of global climate changes (Krizek, 1986; Teramura, 1983; Tevini and Teramura, 1989). Phytotronics is also involved in one of the most exciting research fields of the age, space travel (Dreschel and Sager, 1989; Barta et al., 1992).

Historical review

The world's largest phytotrons

A year after the opening of the first phytotron in Pasadena (USA) in 1949, a much smaller phytotron, with only six growth chambers was opened in Liège (Belgium). A further four phytotrons were opened in the fifties: in Ottawa (Canada) in 1954, in Leverkusen (Germany) in 1955, in Fukuoka Kyushu (Japan) in 1956 and in Moscow (Russia) in 1957.

The boom years for phytotronics were the sixties and seventies. The most important of the 18 phytotrons constructed in the sixties were those in Holzhausen, Germany (1961), Canberra, Australia (1962), Gif-sur-Yvette, France (1963), Wageningen, the Netherlands (1963), Stockholm, Sweden (1965), Madison, USA (1967), Guelph, Canada (1968), Durham and Raleigh, USA (1968) and Palmerston North, New Zealand (1969). The momentum continued into the seventies, when the new phytotrons included those in Irkoutsk, Russia (1970), Krakow, Poland (1972), Martonvásár, Hungary (1972), Budakalász, Hungary (1975), Lethbridge, Canada (1976), Quedlinburg, Germany (1977), Odessa, Ukraine (1978) and Bernburg, Germany (1979).

Extremely detailed information on the technical parameters and costs of the phytotrons opened prior to 1972 is presented by Bilderling (1975). This work was extended to 1979 by Bernáth et al. (1982), who restricted themselves to technical information.

Naturally, new phytotrons continue to be built. According to Sawatsky (1997) phytotrons equipped with Conviron units have been constructed in recent years in the University of Saskatchewan, Canada (167 units), Dupont

Nambsheim, France (21 units), the University of California, USA (120 units), the University of Joensuu, Finland (13 units) and the Indian Agriculture Research Institute, New Delhi, India.

Manufacturers of plant growth units

The manufacturers of plant growth units can be divided into two groups. During the first part of the period under discussion the plant growth chambers were generally built on site to suit the local conditions and requirements. Different firms manufactured the chambers, the climatic equipment, the lighting, etc. Later certain firms included the manufacture of complete plant growth chambers in their profile, while others specialised themselves entirely in this field. Each manufacturer made a characteristic type of chamber, differing in size and technical parameters from those of other firms. Research institutes were thus able to choose the equipment that suited their requirements and their pockets from those available on the market.

Downs (1975) mentioned twenty-four manufacturers, the best-known of which are Controlled Environments Ltd. (Canada), Fisons Scientific Apparatus Ltd., Prestcold Ltd. (UK), Brown Boveri-York Kalte- und Klimatechnik GmbH, Ernst Vötsch Kalte- und Klimatechnik Kg., Karl Weiss Fabrik Elektro-Physikal Geräte (Germany), Koito Industries Ltd. (Japan), Environmental Growth Chambers, Forma Scientific Inc., Hotpack Corp., Percival Manufacturing Co. and Sherer Dual Jet Division (USA).

Langhans (1978) lists a further eleven firms, including Thermotechnique Loubriat (Belgium), Coldstream Ltd. (Canada), Sapratin-Environnement (France), Rubarth and Company (Germany) and Temperature Control Ltd. (New Zealand).

In addition to many of the above, Bernáth et al. (1982) included Secmi S.A. (France), Maschinenfabrik Nema (Germany) and Agrophysical Institute (Russia) among the more important firms.

Naturally the list is not complete and several of the firms mentioned above no longer exist or do not manufacture plant growth equipment any more. Other firms have since come into existence or specialised themselves in this field, including Phoenix Biosystems (Australia), Arctest Ky (Finland), Fitoklima Ltd. (Hungary), Kebo Biomed and Svalöf Weibull AB (Sweden).

Phytotronics literature

Several comprehensive books have been published on the subject in English (Bickford and Dunn, 1972; Downs, 1975; Downs and Hellmers, 1975; Langhans, 1978; Langhans and Tibbitts, 1996), German (Nuernbergk, 1961), Russian (Bondarenko et al., 1978; Rozhdestvenskiy and Klesnin, 1980; Baryshnev, 1984) and Hungarian (Bernáth et al., 1982). The first journal in this field was the Phytotronic Newsletter, edited in the Gif-sur-Yvette (France) phytotron by P. Chouard (later R. Jacques) and N. de Bilderling, 21 issues of which were published between 1971 and 1980. Since 1983 one issue a year of

the journal Biotronics has appeared, edited in the biotron of Kyushu University (Japan) by T. Matsui and later H. Eguchi. A number of large phytotrons publish their own journals, such as the Climate Laboratory Newsletter published in the Palmerston North phytotron since 1973 under the editorship of I.J. Warrington and later E.A. Halligan, or the Phytotron Report published in the North Carolina State University phytotron since 1976 (editors R.J. Downs, later J.F. Thomas). The scientists making use of the phytotron facilities have published their results in tens of thousands of publications in plant research journals.

ASHS Growth Chamber Committee

An important chapter in the history of phytotronics began in 1969, 20 years after the opening of the first phytotron, with the setting up of the American Society for Horticultural Sciences (ASHS) Working Group on Growth Chambers and Controlled Environments at Washington State University in Pullman at the instigation of Theodore Tibbitts. The main aims of this group, which are still valid today, were:

1. To develop guidelines for the measurement and reporting of the environment in controlled environment studies,
2. To publish a procedures manual for growth chamber users,
3. To exchange information.

The first ASHS Growth Chamber Committee was headed by such leading exponents of phytotronics as Wade L. Berry, Robert J. Downs, Donald T. Krizek, Robert W. Langhans and Theodore W. Tibbitts.

The first rough draft of the guidelines for standardising the measurement and reporting of the environment were developed by D. Krizek and T. Tibbitts (Krizek, 1970). These guidelines were approved for general acceptance on an international scale at the Controlled Environments Working Conference in Madison, attended by participants from the USA, Canada, Hungary and Israel (Tibbitts and Kozlowski, 1979). Recently the guidelines were published again in comprehensive form in the book entitled Units, Symbols and Terminology for Plant Physiology (Salisbury, 1996).

The second aim, to publish a procedures manual for growth chamber users, was achieved in 1978 with the publication of "A growth chamber manual" (Langhans, 1978), 2000 copies of which were made available to those working in the field of phytotronics. Technological development and the increase in the demand for this manual led to the publication of a revised version (Langhans and Tibbitts, 1996).

The exchange of information, also included among the original aims, was most effectively served by the annual Meetings (NCR-101 Committee), which provided information not only for the regular attendants, but also for occasional participants, such as the present author, who received the Meeting Minutes. These meetings were complemented by international workshops (Tibbitts and Kozlowski, 1979; Tibbitts, 1994).

The history of the ASHS Growth Chamber Committee, which now covers over a quarter of a century, is described in detail by Tibbitts and Krizek (1997).

The Martonvásár Phytotron

The idea of establishing a phytotron in Martonvásár was first raised in 1959, ten years after the opening of the first phytotron. The exact reproduction of the experimental conditions was essential for the genetic research then underway in the institute. It was also clear that the experimental facilities provided by the phytotron would be an enormous advantage in work on plant physiology, cell biology and breeding.

The Presidium of the Hungarian Academy of Sciences discussed the question of setting up a phytotron in Martonvásár as early as 1961, and agreed on principle that "in order to improve the standard of the basic biological research and plant breeding underway in the institute, an up-to-date phytotron should be built as soon as possible", but it was not until 1970 that the design and construction work was actually begun.

The history of the first quarter-century of the Martonvásár phytotron was detailed by Tischner (1997) at the scientific symposium held to mark the 25th anniversary of the opening of the phytotron.

Parameters

The phytotron was officially opened on November 3rd 1972. The 30 m × 30 m phytotron hall containing the fifty plant growth units is in the centre of the two-storey 50 m × 50 m building. With the exception of the vernalisation chamber and the gradient chamber, all the plant growth units in the Martonvásár phytotron were manufactured by the Canadian firm Controlled Environments Ltd. (Conviron).

The research strategy in the Martonvásár phytotron was elaborated by Rajki (1973). This research strategy aimed to simulate natural conditions in the climatic programmes used for experiments. A method for calculating phytotronic climatic programmes by fitting trigonometric functions to climatic data series measured by meteorologists was elaborated in Martonvásár by Pletser (1973). Naturally, when preparing any specific climatic programme, the technical parameters of the given phytotron unit must also be taken into consideration. The major technical data of the plant growth units in the Martonvásár phytotron were reported by Tischner (1981) and Tischner et al. (1997).

Research

The research underway in the Martonvásár phytotron covers a wide range of topics from plant genetics to breeding. The majority of the experiments are carried out on the plants representing the chief profile of the institute, cereals and maize, but there have always been experiments carried out for other institutes on other plants (e.g. tobacco, sunflower, sugar beet, tomato, paprika, rape, mushrooms, soybeans, carnations, etc.).

The first section of the 1974 Phytotron Operational Rules stated: "The phytotron is a tool for the exact scientific examination of the relationship between metabolism and heredity and for the objective clarification of this cardinal question of biology, more especially of genetics and evolution". In accordance with this aim, during the first ten years of phytotronic research, genetic studies on autumnisation, headed by the then director of the institute, Sándor Rajki, were the major topic (Rajki, 1982, 1985).

The phytotron also provided an excellent background for work on flowering biology, first under the leadership of Erna Rajki and then under that of Beáta Barnabás. This research involved two main fields: flowering biology connected with the development of hybrid wheat, and the possibility of storing the pollen of various cereal species (Barnabás and Rajki, 1976, 1981; Barnabás and Fridvalszky, 1984: Barnabás and Kovács, 1997). Raising plants in a controlled artificial environment meant that interesting, scientifically important questions such as the development of the degree of hydration of pollen compared with the daily rhythm in the field (Barnabás and Rajki, 1976, 1981; Barnabás, 1994) or the operation of the flower opening mechanisms of cytoplasmically male sterile and castrated wheat analogues under various environmental conditions could be investigated (Molnár-Láng et al., 1980).

Beginning in the 1980s, plant cell and tissue culturing methods were gradually included in the research projects. The name of the department was also changed from the Flowering Biology Department to the Plant Cell and Reproduction Genetics Department. Phytotronic facilities played a particularly important role in experiments on wheat anther cultures. It became possible to carry out precise tests on the effect of various abiotic environmental factors (principally temperature) on *in vitro* androgenesis and on spontaneous chromosome doubling. When elaborating *in vitro* selection systems at the haploid and dihaploid levels, the methods developed in the phytotron for testing at plant level were utilised in cell and tissue analyses of cold and frost tolerance. An ever greater part of the research programme is made up of genetic engineering experiments based on plant reproductive processes (Sági and Barnabás, 1989; Barnabás et al., 1991; Barnabás and Kovács, 1992; He et al., 1993; Kovács et al., 1994; Takács et al., 1994; Karimzadeh, 1995; Kovács and Barnabás, 1997). Under phytotronic conditions a large proportion of healthy, vital progeny can be produced from genetically manipulated cells. In the maize dihaploid development programme it is of particular significance that plants of pollen origin can be raised during the winter and the seed of these plants can be tested in field experiments in spring.

Basic research under the supervision of József Sutka has also made up an important part of the phytotron research programme ever since the phytotron was opened. The results achieved include the development of monosomes, auto- and allosubstitutions, the localisation of genes responsible for agronomic properties on wheat chromosomes, and the manipulation of wheat chromosomes (Sutka, 1977, 1979, 1981; Veisz and Sutka, 1989, 1993). Successful work is also in progress on the use of embryo cultures to develop interspecific and

intergeneric hybrids under phytotronic conditions (Sutka, 1980). Since 1982 complex biological research on environmental abiotic stress resistance (frost, drought, heat, salt, herbicide) has been expanded to include both the whole plant and the tissue culture levels. Classical genetic, cytogenetic and biotechnological methods are employed to analyse nuclear and extranuclear gene effects controlling wheat frost resistance, and these genes have been located on the chromosomes (Sutka, 1984; Sutka et al., 1986a, 1986b; Veisz et al., 1987, 1996a; Sutka and Veisz, 1988; Veisz, 1997a, 1997b; Veisz and Sutka, 1998a). The gene pool of frost resistance has been broadened by means of chromosome engineering. An *in vitro* method has been elaborated for the selection of frost resistance (Galiba and Sutka, 1988, 1989). In the early 1990s the *Fr1* frost resistance gene identified by Sutka's team was mapped on the long arm of chromosome 5A by means of RFLP analysis (Sutka, 1994; Galiba et al., 1995).

Galiba et al. (1997) studied the effect of high temperature on the photosynthetic activity and biomass production of bread wheat and durum wheat varieties and found that although the heat stability of cell membranes in durum wheat was better than in bread wheat, the latter was nevertheless more resistant to high temperatures.

Investigations on drought and salt tolerance was begun in the phytotron in the second half of the 80s using wheat tissue cultures induced *in vitro* from immature embryos. Drought was modelled by adding mannitol to the nutrient medium. This substance can be used as an osmotic agent as it binds water and prevents the metabolisation of wheat calli. Calli originating from sensitive and tolerant varieties respond differently to osmotic treatment (Galiba et al., 1989). By analysing the free amino acid composition of the calli it was possible to identify chromosomes bearing genes playing a role in osmoregulation (Galiba et al., 1992). Salt tolerance was modelled by adding common salt to the nutrient medium (Trivedi et al., 1991). The polyamine composition of the calli responded differently to salt (ionic stress) than to mannitol (non-ionic stress) (Galiba et al., 1993a). It follows from this that the polyamine content could be used as a sensitive marker in the determination of drought and salt tolerance.

Hydroponically-grown seedlings of bread wheat varieties differing in drought tolerance were compared under consecutive water (polyethylene glycol, PEG 4000) and salinity stresses. With this method the effect on plants of non-ionic osmotic stress could be studied separately from the toxic effect of NaCl (Nagy and Galiba, 1995).

Investigations carried out by Galiba (1994) indicated that both cold and drought had an influence on the water balance, suggesting that these two forms of stress induced similar physiological changes. One example of this is the phytohormone abscisic acid, which is induced as the result of both cold (Galiba et al., 1993b) and drought (Galiba, 1994). Changes in the sugar metabolism, namely a rise in the soluble carbohydrate content, are also known to occur as the result of both types of stress. With the help of single chromosome recombinant lines it proved possible to map the gene responsible for cold-induced sugar accumulation (Galiba et al., 1997).

Investigations were made on the effect of high and low temperature-induced photoinhibition on various fluorescence induction characteristics (Csapó et al., 1991; Janda et al., 1994a, 1994b), gas exchange parameters (Janda et al., 1998; Galiba et al., 1997), quantitative changes in the contents of certain N-containing compounds (Dőry et al., 1990; Szalai et al., 1997a) and the development of post-chilling symptoms (Szalai et al., 1996; Janda et al., 1996). A relationship was demonstrated between the cold tolerance of crop plants and the characteristics of thermoluminescence curves (Ducruet et al., 1997). It was found that salicylic acid has a protective effect against low temperature stress in young maize plants (Szalai, 1997b).

Detailed studies were made on the effect of low temperature on the metabolism of wheat and maize. It was found that as the result of low temperature quantitative and qualitative changes occurred in polyamine synthesis and in the synthesis and maturation of rRNA (Páldi and Dévay, 1977a, 1977b, 1983; Páldi et al., 1996, 1997; Rácz et al., 1996). It was demonstrated that specific rRNA and protein fractions appeared during vernalisation, a developmental process taking place at low temperature, and that alternative metabolic pathways played a decisive role in polyamine synthesis (Páldi and Dévay, 1977c; Dévay and Páldi, 1983; Lasztity et al., 1991). Certain N-containing compounds (polyamines, amino acids, glycine betaine) and etioplast structures were found to be of great importance in the development of chilling tolerance in maize (Böddi et al., 1997).

Successful research is underway under phytotron conditions on the development of interspecific and intergeneric hybrids with the aid of embryo culture (Lángné Molnár et al., 1985). Studies were made on the effect of temperature and light on fertilisation and embryo development when crossing wheat and barley (Molnár-Láng and Sutka, 1994). The hybrids created in this way were multiplied in tissue culture and raised in the phytotron (Molnár-Láng et al., 1991, 1996a). The *kr1* allele responsible for crossability was incorporated into the Mv 9 wheat variety (Molnár-Láng et al., 1996b) and this line was used in a number of combinations to develop interspecific hybrids under phytotron conditions (Farshadfar et al., 1994; D. Nagy et al., 1998).

The phytotronic method of frost resistance testing elaborated by Erna Rajki (1980) and later by Ottó Veisz for the genetic investigation of frost resistance can also be applied with excellent results for frost resistance testing in wheat breeding (Bedő et al., 1987; Veisz and Rajki, 1987; Szunics et al., 1987, 1998; Veisz, 1989; Veisz et al., 1997, 1998; Veisz and Sutka, 1998b). Not only wheat breeders from Martonvásár, but also officials from the Hungarian Institute for Agricultural Quality Control set up experiments each year in the Martonvásár phytotron to test the frost resistance of new varieties. The results of these tests are then used in deciding whether the new varieties should be registered.

A special phytotronic wheat breeding strategy has been elaborated, based on the creation of a large number of combinations and on the raising of four generations a year. During the seedling stage the plants are kept in a vernalisation chamber for six weeks. At the end of the vernalisation period the

plants are transferred to plastic pots and kept on a spring climatic programme for a further six weeks until they reach the heading stage. The three-week flowering period is followed by a three-week ripening stage. This means a total of six weeks of vernalisation and three months of plant growth for each generation. In other words, the same vernalisation chamber and growth benches can be used to raise as many as four generations a year (Balla, 1980, 1982, 1983).

In order to perfect the method, the institute's wheat breeders have conducted phytotronic experiments to study the germination of grains harvested prior to full ripening, the effect of daylength and temperature on the development and ear fertility of winter wheat varieties, and the photoperiodic sensitivity of wheat varieties (Bedő and Balla, 1980; Balla, 1983; Láng and Balla, 1988).

The maize research division of the institute makes constant use of the phytotron facilities, but only uses approximately 5% of the total capacity. Mention should be made of the cold tests (Szundy and Kovács, 1981a, b; Herczegh and Marton, 1986; Qhang and Szundy, 1989), studies on the cold wave tolerance of *Opaque-2* maize inbreds and their normal analogues (Gupta and Kovács, 1974, 1975, 1976) and on the temperature dependence of emergence and initial development (Marton, 1990, 1991), tests on sensitivity to various herbicides (Berzsenyi et al., 1985, 1986, 1989) and growth programmes set up in the winter to improve the efficiency of maize breeding. In these latter experiments the aim is to raise valuable genotypes from seed to seed, carrying out the most important crosses, and thus increasing the number of generations which can be raised per year.

In addition to the natural variability of the earth's climate, global climate changes can now be observed as the result of the increased greenhouse effect. The plant growth chambers found in phytotrons provide an excellent opportunity for the exact investigation of the effect of the expected rise in atmospheric temperature and reduction in rainfall on the growth and development of plants (Veisz et al., 1996c). In this connection, various experiments have been carried out in Martonvásár on drought tolerance (Galiba et al., 1989; Galiba, 1994; Farshadfar et al., 1995).

Over the past few decades the quantity of carbon dioxide emitted into the earth's atmosphere due to human activities has increased to such an extent that its concentration in the troposphere is now over 25% higher than it was prior to the industrial revolution (350–380 μmol mol^{-1}, compared with 280 μmol mol^{-1}). This represents a rise of some 0.45% a year, which can be measured on all parts of the globe (Keeling et al., 1976a,). The probable effects of a further increase in the atmospheric carbon dioxide concentration have also been studied in the Martonvásár phytotron (Veisz and Tischner, 1995; Veisz et al., 1996b; Harnos et al., 1998).

When assisting in the establishment of the phytotron and in its recent reconstruction, the Hungarian Academy of Sciences wished not only to improve the research facilities available to the Martonvásár institute, but also to create exact research conditions for the whole of Hungarian plant research. Thus, the

research facilities available in the Martonvásár Phytotron are constantly open to all plant researchers. In the course of the years, experiments have been set up for dozens of other institutions, including foreign ones. In some cases this meant a single experiment, while in other cases it involved a whole series of experiments over a number of years.

Further information on the scientific research underway in the Martonvásár phytotron is to be found in the volume of papers presented at the international symposium organised to celebrate the 25th anniversary of the opening of the phytotron (Bedő et al., 1997).

Gradient chamber

Among the technical developments achieved in the Martonvásár phytotron, the most significant was the construction of a gradient plant growth chamber, patented by the inventors not only in Hungary but in a number of developed countries (Canada, Germany, Japan and the USA) (Horváth et al., 1978). On the gradient growth bench two gradients perpendicular to each other can be created for the environmental factors chosen. In this way a large number of combinations of these two factors is produced in a single growth unit, at the same time, while the other factors remain constant. If the gradient values are well chosen, a single experiment may be sufficient to determine the optimum values for the two factors in question, thus leading to considerable savings in energy, time and experimental material. The method has the further advantage that the duration of the gradient effects can be programmed as a third variable (Tischner and Veisz, 1996).

Conclusions

It is now obvious to plant scientists that the process initiated by Went fifty years ago with the opening of the first phytotron in Pasadena is not only characterised by a glorious past and prosperous present, but can also be expected to continue to develop in the future, since without the programmability and reproducibility of the environmental conditions which can be achieved in phytotronic plant growth units exact plant research would be impossible. At the same time, the number of problems waiting to be solved by plant scientists is on the increase. As technology develops there will be further improvements in the standard of the parameters in phytotron research units, making them suitable for the solution of research tasks for which the present equipment is not sufficiently advanced. One example of this could be the cross-gradient growth bench, which is likely to prove indispensable for optimisation purposes once the technical design has been perfected.

I should like to close with a quotation on the future of phytotronics made by Ted Tibbitts (1997), one of the most prominent leaders in this field, at the conference in Martonvásár: "The challenges in phytotronics are very large and exciting. Phytotron facilities are needed and I believe they will continue to be centres of research activity around the world for several more decades."

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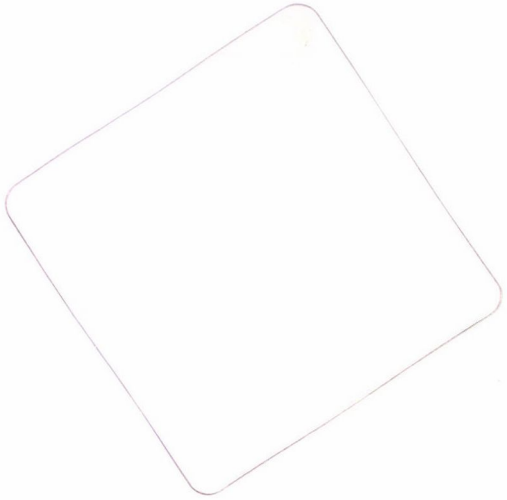

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SHORT COMMUNICATION

REVIEWS

Acta Agronomica Hungarica, 47(2), pp. 109–116 (1999)

GENETIC ANALYSIS OF PHENOTYPIC STABILITY PARAMETERS IN WHEAT (*TRITICUM AESTIVUM* L.)

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The genetic properties of different types of stability parameters for individual genotypes were investigated using an eight-parent half-diallel cross in a randomized complete block design with three replications. Data pertaining to the parents and F_1s were subjected to Griffing and Hayman methods for the genetic analysis of eight types of stability statistics. The results of analysis of variance showed that genetic variation exists for almost all the stability parameters. Moderate heritability estimates were observed for coefficient of variation (CVi) and superiority measure (Pi). The estimates of variance components suggested that genes controlling environmental variance (S^2i), regression coefficient (bi), ecovalence (W^2i) and Pi are predominantly additive, while non-additive gene action was predominant for CVi, stability variance (σ^2i) and coefficient of determination (R^2i). The results of combining ability effects indicated that the best general combiners for the improvement of adaptation were Chinese Spring and Shakha, while the best stable specific combination was Shakha/Kobomugi.

Key words: diallel crosses, phenotypic stability, gene action, heritability, combining ability

Introduction

In any plant breeding programme it is necessary to screen and identify phenotypically stable genotypes which can perform uniformly under different environmental conditions. Such a breeding effort requires basic information on genotype-environment interaction (Miezan et al., 1979; Kumar and Chowdhury, 1991; Gauch et al., 1997).

There are several concepts of stability and statistics that can be used to estimate the stability of genotype performance. The concepts, formulas and relationships were reviewed by Lin et al. (1986), Westcott (1986), Becker and Leon (1988), Crossa (1990), Lin and Binns (1994).

Irrespective of how a stability parameter is defined, one of the most critical questions is whether it is genetic. If the characteristic measured by the parameter is non-genetic, it is not heritable and thus selection for such a parameter is fruitless (Lin and Binns, 1991; 1994).

To claim that a stability measure is genetic, the parameter must be examined with respect to the progenies of crossed material (Lin and Binns, 1991).

In the present investigations the genetic properties of different types of stability indices were studied using a set of diallel cross data.

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Materials and methods

An eight-parent half-diallel cross was made in the Agricultural Research Institute of the Hungarian Academy of Sciences during the year 1993. All crosses were made using hand emasculation. Both parents and F_1s were grown under irrigated and rainfed conditions for two years in the nursery of the College of Agriculture, Razi University, Kermanshah, Iran.

The experimental material was seeded in a randomized complete block design with three replications in three-row plots, 15 m in length with rows spaced 25 cm apart in the field. Eighteen seeds per plot were planted. In 1996 the parents (Plainsman=PLS, Chinese Spring=CS, Cappelle Desprez=CD, Regina=REG, Shakha=SAK, Kobomugi=KOB, Saberbeg=SAB and Karchia=KAR) and the F_1s were grown under dryland agriculture in the same place and with the same seeding rate as in the first year (1995). Disease and insect incidence were very low and the climate conditions were near normal in both years. From each entry (parents and F_1s) five competitive plants were randomly selected from each replication in both the years for recording observations on grain yield/plant.

Statistical methods

Data pertaining to the parents and F_1s, i.e. n(n+1)2=8(8+1)/2=36 total entries were subjected to Griffing model I, method II and the Hayman method (Mather and Jinks, 1982; Kearsey and Pooni, 1996).

Different phenotypic stability parameters were calculated, including: environmental variance (S^2i) (Petersen, 1994), genotypic coefficient of variation (CVi) (Francis and Kannenberg, 1978), Wricke's (1986) ecovalence (W^2i), Shukla's (1972) stability variance (σ^2i), regression coefficient (bi) (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966), deviation from regression (S^2di) (Eberhart and Russell, 1966), coefficient of determination (R^2 i) (Pinthus, 1973) and superiority measure (Pi) (Lin and Binns, 1988).

Results

The results of analysis of variance for the various stability criteria are presented in Table 1.

The analysis of variance revealed that all 36 genotypes had highly significant differences for all the stability parameters except S^2di, so this was omitted from the further analysis.

Data obtained from the combining ability analysis of Griffing (1956) for the eight phenotypic stability parameters are presented in Table 2.

The mean squares due to general combining ability (GCA) and specific combining ability (SCA) were highly significant for all the stability parameters, except the GCA for CVi and R^2i.

The estimates of GCA and SCA effects for the eight stability parameters under consideration, presented in Tables 3 and 4 are detailed as below:

1. Environmental variation (S^2i)

Among the parents, PLS and REG had highly significant positive GCA effects, while SAB and KAR showed highly significant negative GCA effects. CD, CS, SAK and KOB had non-significant GCA effects.

Table 1
Analysis of variance of phenotypic stability parameters

Source of variance	Mean square of stability parameters							
	d.f.	S^2i	bi	S^2di	W^2i	CVi	σ^2i	R^2i	Pi
Genotypes	35	368.23**	0.37**	31.23ns	70.94**	548.18**	18.74**	0.017**	426.23**
Replication	2	4.99**	0.10ns	1.021ns	77.163**	473.5*	17.15*	0.006ns	1221.55**
Error	70	33.23	0.038	1.97	13.66	107.8	4.02	0.0056	45.99
Total	107	149.85	0.145	2.3	33.58	258.69	6.3	0.00093	192.34

ns = non-significant

Table 2
Combining ability analysis of stability parameters

Source of variance	Mean square of stability parameters							
	d.f	S^2i	bi	W^2i	CVi	σ^2i	R^2i	Pi
GCA	7	79.95**	0.0951**	11.21*	69.33ns	6.22**	0.0034ns	238.7**
SCA	28	133.44**	0.129**	26.75**	211.07	6.97**	0.0062**	117.9**
Error	70	11.076	0.0125	4.55	35.94	1.34	0.0019	15.3

ns = non-significant

Table 3
Estimates of GCA effects in an eight-parental diallel experiment for different phenotypic stability parameters

Genotypes	Phenotypic stability parameters						
	S^2i	bi	W^2i	CVi	σ^2i	R^2i	Pi
PLS	+4.76**	+0.17**	+0.06ns	+3.18ns	+0.09ns	+0.03*	–5.63**
REG	+2.06*	+0.06ns	+0.81ns	+2.1ns	+0.44ns	–0.006ns	–0.87ns
CD	+0.108ns	+0.02ns	–1.31*	+1.05ns	–0.51ns	+0.0002ns	–3.65**
CS	–0.28ns	+0.019ns	–1.79**	–2.02ns	–0.82*	+0.02ns	–3.65**
SAK	–0.27ns	–0.019ns	+0.44ns	–4.5ns	+0.305ns	–0.024ns	–2.838
SAB	–2.68**	–0.102**	+0.93ns	+1.45ns	+0.56ns	–0.025*	+7.34**
KAR	–4.55**	–0.15**	–0.23ns	–2.32ns	+0.125ns	+0.009ns	+7.43**
KOB	+0.83ns	+0.009ns	+1.08ns	+1.05ns	+0.58ns	+0.0001ns	+0.26ns

ns = non-significant

The crosses KOB/PLS, REG/PLS, REG/CS, REG/KOB, CD/CS, CD/KAR, KOB/CD, CS/SAK and CS/KAR had highly significant positive SCA effects. SAK/PLS, SAB/PLS, KAR/PLS, REG/SAK, CD/SAK, CS/KOB, SAB/KOB and KAR/KOB showed highly significant negative SCA effects. All other crosses revealed non-significant SCA effects. The poorest specific combiner, with a highly significant positive SCA effect, was KOB/CD, while the best specific combination, with a highly significant negative SCA effect, was REG/SAK.

Table 4
Estimates of SCA effects in an eight-parental diallel experiment for different phenotypic stability parameters

Crosses	Phenotypic stability parameters						
	S^2i	bi	W^2i	CVi	σ^2i	R^2i	Pi
CS/PLS	+3.8ns	+0.84ns	0.30ns	1.55ns	0.08ns	–0.01ns	–6.01ns
SAK/PLS	–8.2**	–0.23	–2.6ns	–16.2**	+0.001ns	+0.001ns	+0.18ns
SAB/PLS	–7.3**	–2.28**	–1.1ns	–2.44ns	–0.14ns	–0.014ns	+11.35**
KAR/PLS	–7.3**	0.19*	–4.1*	–13.02**	–2.31*	+0.04ns	–1.30ns
KOB/PLS	+10.3**	+0.38**	+0.389ns	+0.52ns	+0.08ns	+0.04ns	–15.44**
REG/PLS	+11.08**	+0.39**	+1.8ns	+22.1**	+0.88ns	–0.013ns	–7.80*
REG/CS	+21.3**	+0.54**	+11.5**	+12.1*	+6.02**	–0.001ns	–10.71**
REG/SAK	–13.1**	–0.58**	+6.6*	–6.14ns	+3.41**	–0.25**	+2.61ns
REG/SAB	–3.7ns	–0.084ns	–3.8*	+0.18ns	–2.01*	+0.04ns	–6.08*
REG/KAR	–4.2ns	–0.112ns	–2.5ns	–14.7**	–1.47ns	+0.007ns	–9.11**
REG/KOB	+14.3**	+0.48**	+1.6ns	+15.6**	+0.76ns	+0.08*	+28.7**
CD/PLS	+2.5ns	+0.18*	–4.54**	+17.66**	–2.42**	+0.0024ns	–1.47ns
CD/CS	+23.8**	+0.64**	+10.5**	+23.66**	+5.6**	+0.025ns	–12.44**
CD/SAK	–8.7**	–0.054ns	+0.64ns	–0.51ns	+0.89ns	–0.14**	+11.86**
SAB/CD	–1.23ns	+0.26**	+0.93ns	+13.40ns	–1.38ns	–0.035ns	–7.9*
CD/KAR	+5.93*	+0.77**	–2.11ns	+2.99ns	+5.2**	+0.075*	–18.9**
KOB/CD	+26.8*	+0.075ns	+10.3**	+21.5**	–1.45ns	+0.053ns	+2.2ns
CD/REG	+0.62ns	–0.32**	–2.4ns	+13.4**	+0.16ns	+0.010ns	+12.84**
CD/SAK	+16.3**	+0.47**	+6.2**	+24.69**	+3.1**	+0.048ns	–4.13ns
CD/SAB	–2.50ns	–0.027ns	–3.21ns	–1.63ns	–1.9*	–0.022ns	–1.21ns
CS/KAR	+5.6*	+0.21*	–1.11ns	–2.14ns	–0.72ns	+0.010ns	–14.45**
CS/KOB	–10.4**	–0.34**	–0.54ns	–17.31**	–0.43ns	+0.09**	+6.54*
SAK/SAB	–4.7ns	–0.12ns	–4.12*	–0.23ns	–1.67ns	+0.05ns	+5.08ns
SAK/KAR	–0.59ns	–0.010ns	–2.8ns	–1.43ns	–1.55ns	+0.07*	–2.6ns
SAK/KOB	–2.56ns	0.002ns	–6.08**	+3.2ns	–3.2**	0.056ns	+0.25ns
SAB/KAR	–2.65ns	–0.046ns	–2.47ns	–0.49ns	–1.37ns	+0.005ns	+0.77ns
SAB/KOB	–5.93*	–0.14ns	–4.92**	–14.62**	–2.7**	+0.037ns	–6.33*
KAR/KOB	–6.96**	–0.22*	–1.604ns	–17.07**	–0.86ns	–0.011ns	–1.98ns

ns = non-significant

2. Regression coefficient (bi)

PLS revealed a highly significant positive GCA effect and SAB and KAR showed highly significant negative GCA effects. The rest of the parents showed non-significant GCA effects. The crosses KOB/PLS, REG/CS, REG/KOB, CD/PLS, CD/CS, SAB/CD and CD/KAR had highly significant positive SCA effects. SAK/PLS, SAB/PLS, KAR/PLS, REG/SAK, CS/SAK, CS/KOB and KAR/KOB displayed highly significant negative SCA effects. The rest of the combinations showed non-significant SCA effects.

3. Ecovalence (W^2i)

CD and CS revealed highly significant negative GCA effects, while the other parents had non-significant GCA effects. The crosses REG/CS, REG/SAK, CD/CS, KOB/CD and CS/SAK had highly significant positive SCA effects, while KAR/PLS, REG/SAB, CD/PLS, SAK/SAB, SAK/KOB and SAB/KOB showed significant and highly significant negative SCA effects. The rest of the crosses displayed non-significant SCA effects.

4. Genotypic coefficient of variation (CVi)

CS had a significant negative GCA effect and the rest of the parents exhibited non-significant GCA effects. The crosses REG/PLS, REG/CS, REG/KOB, CD/PLS, CD/CS, SAB/CD, KOB/CD, CD/REG and CS/SAK showed highly significant positive SCA effects, while SAK/PLS, KAR/PLS, REG/KAR, CS/KOB, SAB/KOB and KAR/KOB revealed highly significant negative SCA effects. The rest of the combinations had non-significant SCA effects.

5. Stability variance (σ^2i)

CS had a significant negative GCA effect and the rest of the parents revealed non-significant GCA effects. Among the hybrids REG/CS, REG/SAK, CD/CS, CD/KAR and CD/REG exhibited highly significant positive SCA effects, while KAR/PLS, REG/SAB, CD/PLS, CS/SAK, SAK/KAR and SAB/KOB displayed highly significant negative SCA effects. The rest of the crosses showed non-significant SCA effects.

6. Coefficient of determination (R^2i)

Among the parents PLS showed a significant GCA effect and SAB had a significant negative GCA effect. REG/KOB, CD/KAR, SAK/SAB and SAK/KOB revealed significant positive SCA effects, while REG/SAK and CD/SAK displayed significant negative SCA effects. The rest of the combinations had non-significant SCA effects.

7. Superiority measure (Pi)

SAB and KAR exhibited highly significant positive GCA effects, while PLS, CS and SAK had highly significant negative SCA effects. The other parents showed non-significant GCA effects. Among the combinations SAB/PLS, REG/SAK, CD/SAK, CD/REG and CS/KOB revealed highly significant positive SCA effects, while KOB/PLS, REG/PLS, REG/CS, REG/SAB, REG/KAR, CD/CS, SAB/CD, CD/KAR, CS/KAR and SAB/KOB displayed highly significant negative SCA effects. The rest of the crosses had non-significant SCA effects.

The results of the genetic components of variation are presented in Table 5.

Table 5
Analysis of genetic components of variation, narrow sense heritability (Hn) and average degree of dominance (a*) in an 8 × 8 diallel

Source of variance	Mean square of phenotypic stability parameters							
	d.f	S^2i	bi	W^2i	CVi	σ^2i	R^2i	Pi
a	7	79.95**	0.095**	11.21*	69.3ns	2.65ns	0.034ns	238.7**
b1	1	448.9**	0.52**	0.13ns	672.2**	0.062ns	0.011*	332.5**
b2	7	35.6**	0.05**	28.86**	154.2**	8.01**	0.009*	62.85**
b3	20	151.9**	0.14**	27.35**	207.9**	7.5**	0.005**	126.5**
Error	70	11.07	0.0125	4.45	35.9	1.34	0.002	15.33
Hn(%)		7.4	5.1	10	12	2.1	8.2	17
a*		3.4	4.1	2.7	2.5	6.1	2.8	2.1

ns = non-significant

The analysis of variance for the genetic components revealed that the additive effect (a) of the genes controlling S^2i, bi, W^2i and Pi is highly significant, while this item is non-significant for CVi and R^2i. The direction of dominance (b1) is highly significant for S^2i, bi, CVi, R^2i and Pi , and non-significant for W^2i and σ^2i.

The homogeneity of the dominance effect (b2) is highly significant for all the phenotypic stability parameters except S^2di, which is non-significant. The part of dominance deviation that is unique to each F_1 (b3) is highly significant for all the phenotypic stability parameters.

Discussion

A basic knowledge of the genetics and nature of the gene action controlling phenotypic stability is a pre-requisite for breeders in manipulating materials for the isolation of superior lines.

The results of analysis of variance showed that genetic variation exists for all the stability statistics, indicating that selection for these parameters may be effective in the improvement of adaptation. However, the selection efficiency is related to the magnitude of heritability. The estimates of heritability were grouped in three categories: low (less than 10%), moderate (10 –30%) and high (greater than 30%) (Nematzadeh, 1987; Farshadfar, 1998). Moderate heritability estimates were observed for S^2di, CVi and Pi. The fact that the heritability of the stability parameters is low suggests that environmental effects constitute a major portion of the phenotypic variation in these adaptability criteria.

Combining ability analysis partitions the genotypic variability into variances due to GCA and SCA, which represent additive and dominance effects. In the present case the variances due to GCA and SCA were significant for all the parameters, indicating the presence of both additive and non-additive genetic variance in the expression of all stability indices. However, the estimates

of variance components suggested that genes controlling bi, W^2i and Pi are predominantly additive, while non-additive gene action was predominant for CVi, σ^2i and R^2i.

Since CVi, σ^2i and R^2i were influenced by non-additive gene action, selection for these stability parameters would have to be delayed until the F_3 or F_4 generation in a conventional hybridization programme. Such a delay would permit a loss of non-additive genetic variance through inbreeding, so that the additive genetic variance could be more clearly evaluated.

The significance of the b1 item for S^2i, CVi, R^2i and Pi indicates that the dominance deviation of the genes controlling these stability criteria is predominantly in one direction, that is, i.e., a directional dominance effect. The significant b2 for almost all the stability parameters implies the asymmetry of gene distribution, i.e. some parents contain more dominant alleles than the others.

The significant b3 for all the stability parameters shows that there are dominance effects specific to individual crosses. This item is equivalent to the specific combining ability of Griffing (1956) for a fixed model where the inbred lines are omitted from the analysis. The average degree of dominance (a*) revealed the existence of overdominance for all the stability parameters. As this kind of gene action is accompanied by hybrid vigour or heterosis, it suggests the exploitation of heterosis breeding for adaptability.

With regard to the combining ability analysis for all the stability parameters, the best general combiner for improving adaptability is CS, followed by SAK, while the best specific combination for improving adaptability is SAB/KOB, followed by KAR/PLS and KAR/KOB.

The predominance of additive effects in the inheritance of linear responses was reported by Eberhart and Russell (1966; 1969) and Patanothai and Atkins (1974). Dhillon and Singh (1977) concluded that both additive and non-additive effects were involved in the inheritance of deviation from regression.

Busch et al. (1976), however, indicated that deviation from regression was simply inherited and could be predicted from the parents. In contrast, Patanothai and Atkins (1974) concluded that the inheritance of deviations from regression was controlled predominantly by GCA, whereas both GCA and SCA were equally important in the expression of deviation. Lin and Binns (1988) showed that type 1 (S^2i, bi, CVi) and type 4 (Pi) were additive, while type 2 (bi, W^2i, σ^2i) and type 3 (S^2di) were not.

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ENZYMATIC STUDIES IN SALT-TOLERANT AND SALT-SUSCEPTIBLE RICE CULTIVARS UNDER THE INFLUENCE OF HYDROXYPROLINE AND NaCl

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The effect of hydroxyproline (HP) and NaCl on the growth, proline content and *in vitro* activity of enzymes involved in various parts of the metabolism was studied in callus cultures of salt-tolerant and salt-susceptible rice cultivars. The HP-adapted callus of the salt-tolerant variety Bhoora rata exhibited greater dry weight and proline content, stimulated activities of cellulase, invertase and amylase, and decreased activities of proline and IAA oxidases in response to NaCl as compared to its salt control, and also when compared to the salt-sensitive calli of the variety GR_{11}. The variation in the levels of these enzymes under the influence of HP could possibly act in improving the salt tolerance mechanism of rice cultivars when growth was affected by a threshold level of salinity.

Key words: rice, callus, hydroxyproline, NaCl, enzymes

Introduction

Salinity is a major osmotic stress limiting plant growth and productivity (Skriver and Mundy, 1990). Plant tissue culture techniques have been employed successfully to develop salt-tolerant lines. The unique advantages, the methodologies and accomplishments of the *in vitro* production of salt-tolerant plants have been reviewed by many authors (Tal, 1993; Dix, 1993). In rice, the accumulation of proline has been implicated to confer salt tolerance (Bhattacharya, 1991). There is also evidence that cell lines resistant to hydroxyproline (HP - toxic proline analogue) are tolerant to NaCl (Van Swaaij et al., 1986). The basic principle is that the resistance of a cell line to a toxic amino acid analogue is caused mostly by overproduction of the corresponding amino acid (Maliga, 1984). Proline-overproducing cell lines were found to be salt-tolerant in many species (Shevyakova et al., 1994, Vazquez-Flota and Loyola-Vargas, 1994). The activity of a number of key enzymes was shown to be increased or decreased by NaCl salinity (Gosett et al., 1994, Rus Alvarez and Guerrier, 1994). Carbon metabolism is one of the important factors determining the salt tolerance phenotype (Cushman et al., 1990). Amylase, cellulase and invertase are known to hydrolyse complex carbohydrates into simpler sugars. The accumulation of sugars has also been shown to act as an osmoticum under NaCl stress (Sacher and Staples, 1985). The disruption of plant growth and development by salinity is mainly due to the hormonal imbalance (Levitt, 1990). The exogenous application of IAA could counteract the negative effects of salinization (Darra and Saxena, 1973). The activity of IAA oxidase can therefore be taken as a reflection of the endogenous level of IAA. Thus, the present

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investigation was undertaken with a view to getting greater insight into the mechanism of salt tolerance in rice, as related to the level of proline and the activity of amylase, cellulase, invertase, proline and IAA oxidases.

Materials and methods

Certified seeds of two rice cultivars, namely Bhoora rata (BR-salt-tolerant) and GR_{11} (salt-susceptible) were obtained from Gujarat Agricultural University, Nawagam, Gujarat, India. Callus was initiated by inoculating the dehusked seeds on LS medium (Linsmaeir and Skoog, 1965) containing 2.5 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) at pH 5.8. Regular subculture of the callus was done at intervals of two weeks. HP-adapted cell lines of rice were isolated by culturing the callus on LS medium containing LD_{50} concentration of HP (10 mM). After two weeks of incubation these calli were transferred to LS medium containing LD_{50} concentration of NaCl (200 mM) and the growth of the tissues and their levels of proline, amylase, cellulase, invertase, proline and IAA oxidases were determined at the end of 0, 2, 4 and 6 weeks of incubation. Growth was measured as dry weight accumulation.

The proline level was determined by the colorimetric method described by Bates et al. (1973).

The enzymes were extracted according to the method followed by Chanda et al. (1986). A known weight of callus was homogenised in cold 0.2 M borate buffer (pH 8.6), along with a pinch of glass powder, for 5 minutes using a chilled mortar and pestle. The homogenate was centrifuged at 10,000×g for 10 minutes at 0°C. Chilled acetone at a ratio of 1:2 (v/v) at 0°C was added to the extract to precipitate the protein present. The precipitated protein was sedimented by centrifugation at 15,000×g for 15 minutes at 2°C and was dissolved in different buffers for various enzyme assays.

The method of Huang and Cavalieri (1979) was followed for the assay of proline oxidase. One unit of enzyme is defined as the amount of enzyme which causes a change in optical density of 0.01 per minute at 600 nm under the assay conditions and the activity is expressed as units per mg protein.

The method of Bernfeld (1955) was employed for determining the total amylase activity. One enzyme unit is defined as the amount of enzyme required to liberate 100 μg of maltose per 30 minutes.

The activity of acid invertase was measured by estimating the reducing sugars produced in the assay system after an incubation period of 30 minutes. The assay system (2 ml) contained 1.0 ml 0.1 M acetate buffer (pH 4.6), 0.5 ml of 2% sucrose and 0.5 ml of enzyme extract. The reaction mixture was incubated for 30 minutes at 30±1°C and the reaction was terminated by keeping the tubes in a boiling water bath for 10 minutes. An aliquot from the reaction mixture was used for the estimation of reducing sugars according to the procedure of Somogyi (1952). The enzyme unit is defined as the amount of enzyme required to produce 100 μg of glucose per 30 minutes under the assay conditions and the enzyme activity is expressed as units per mg protein.

For cellulase assay, the method followed by Trivedi and Rao (1979) was employed with certain changes. The assay system contained 1.0 ml of 0.05 M acetate buffer (pH 5.0), 0.5 ml of enzyme and 0.5 ml of 1% carboxymethyl cellulose (CMC, Sigma), prepared in 0.05 M acetate buffer (pH 5.0). The reaction mixture was incubated at 37°C for 30 minutes. The reaction was terminated by heating the tubes in a boiling water bath for 5 minutes and an aliquot of the reaction mixture was used to determine the reducing sugars released according to the method of Somogyi (1952). One enzyme unit is defined as the amount of enzyme required to release 100 μg of reducing sugars per 30 minutes under the assay conditions and the activity is expressed as units per mg protein.

IAA oxidase activity was determined by the method of Gordon and Weber (1951). One enzyme unit is defined as the amount of enzyme required to oxidise 100 μg of IAA per 30 minutes under the assay conditions and the activity is expressed as units per mg protein.

The experiments were repeated three times with five replicates each. The data were analysed by analysis of variance and the means were separated by the least significant difference test.

Results and discussion

The growth of calli under all treatments followed a typical pattern, registering maximum growth at the end of the fourth week. The incorporation of 200 mM NaCl in the medium led to a significant reduction in the dry weight of the calli of both rice cultivars at the end of the fourth week (50% and 42% reduction in the dry weights of BR and GR_{11}, respectively). A similar pattern for the growth of calli in *Lycopersicon penelli* (tolerant) and *L. esculentum* (susceptible) has been reported by Guerrier and Bourgeias Chaillou (1994) under NaCl stress. When cultured on saline medium HP-exposed cells showed better growth compared to the salt control. The addition of HP to the saline medium further improved the growth of HP-exposed cells by 33% and 40%, respectively, in BR and GR_{11} (Table 1).

The free proline content of both rice cultivars rose progressively from the second week, reaching the maximum level at the end of the sixth week (Table 2). It is interesting to note that the free proline content of the control calli of both rice cultivars was low prior to the imposition of either NaCl or HP treatment. The most significant changes in proline content took place at the end of the sixth week in HP-exposed calli grown on medium containing both HP and NaCl. The proline level increased to a maximum of 5 times that of the control in BR callus, accounting for a maximum 4.3% of the callus fresh weight. In GR_{11} callus the level of proline was nearly 6 times higher as compared to the control, accounting for a maximum of 2.7% callus fresh weight. An increase in the proline level in response to NaCl has been reported by many workers (Prakash and Sarin, 1993). In addition to acting as an osmoprotectant, proline also serves as a sink for

Table 1
Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on growth (mg dry weight) of rice callus in varieties BR and GR_{11}

Treatments	Variety	Culture duration (weeks)			
		0	2	4	6
Control	BR	19.0 ± 0.4*	38.0 ± 0.6[b]	72.0 ± 0.6	70.0 ± 1.4
	GR_{11}	20.0 ± 0.9	40.0 ± 1.0	60.0 ± 1.1	60.0 ± 3.5
HP	BR	19.0 ± 0.4	36.0 ± 0.9	41.0 ± 0.9[a]	41.0 ± 0.9[a]
	GR_{11}	20.0 ± 0.9	30.0 ± 0.7	32.0 ± 0.6	32.0 ± 0.6
NaCl	BR	13.0 ± 0.5	26.0 ± 0.7	42.0 ± 0.9[a]	41.0 ± 0.9[a]
	GR_{11}	18.0 ± 1.0	27.0 ± 0.7	30.0 ± 1.0	30.0 ± 1.0
NaCl in HP-exposed cells	BR	16.0 ± 0.5	34.0 ± 1.3[b]	50.0 ± 0.9	49.0 ± 1.0
	GR_{11}	18.0 ± 1.0	29.0 ± 0.8[a]	39.0 ± 1.3	38.0 ± 1.6
NaCl+HP in HP-exposed cells	BR	16.0 ± 0.4	38.0 ± 0.7	56.0 ± 1.3	54.0 ± 1.3
	GR_{11}	18.0 ± 0.9	29.0 ± 0.7[a]	42.0 ± 1.2[a]	42.0 ± 1.2[a]

Values in the same column with the same superscript do not differ significantly ($P < 0.05$) according to the LSD test

* Values indicate ± Standard deviation of three separate measurements

Table 2
Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on the free proline content (mg g^{-1} fresh weight) of rice callus in varieties BR and GR_{11}

Treatments	Variety	Culture duration (weeks)			
		0	2	4	6
Control	BR	4.8 ± 0.3*	1.8 ± 0.4	7.5 ± 0.7[a]	9.1 ± 0.5[a]
	GR_{11}	5.6 ± 0.2	2.9 ± 0.6[a]	3.9 ± 0.5	4.4 ± 0.6
HP	BR	4.8 ± 0.3	5.9 ± 0.7[d]	21.2 ± 0.4	27.5 ± 0.6[b]
	GR_{11}	5.6 ± 0.2	6.0 ± 0.4[d]	6.6 ± 0.3[a]	9.0 ± 1.2[a]
NaCl	BR	1.8 ± 0.4	2.8 ± 0.3[a]	15.0 ± 1.1[b]	18.3 ± 1.0
	GR_{11}	2.9 ± 0.6	2.9 ± 0.3[a]	14.4 ± 0.2[b]	14.8 ± 1.0
NaCl in HP-exposed cells	BR	5.9 ± 0.7	3.5 ± 0.3[b]	35.2 ± 0.9	38.1 ± 0.8
	GR_{11}	6.0 ± 0.4	3.5 ± 0.7[b]	18.5 ± 0.8	20.3± 0.3
NaCl+HP in HP-exposed cells	BR	5.9 ± 0.7	4.3 ± 0.5[c]	39.6 ± 1.2	43.4 ± 1.4
	GR_{11}	6.0 ± 0.4	4.1 ± 0.3[c]	22.5 ± 1.4	26.6 ± 0.8[b]

Values in the same column with the same superscript do not differ significantly ($P < 0.05$) according to the LSD test
*Values indicate ± Standard deviation of three separate measurements

energy to regulate redox potentials (Saradhi and Saradhi, 1991), as a hydroxy radical scavenger (Smirnoff and Cumbes, 1989), as a solute that protects macromolecules against denaturation (Schobert and Tschesche, 1978), and as a means of reducing the acidity in the cell (Venekamp et al., 1989).

The activity of proline oxidase decreased from the second week onwards in the calli of both rice cultivars. In salinized cells of BR the proline oxidase activity showed a significant decrease of nearly 41%, while in GR_{11} salinized cells the decrease was 15%. The activity of proline oxidase further decreased in calli which were pre-exposed to HP and then grown on medium containing both HP and NaCl (Table 3). A decrease in proline oxidase activity under salt stress has been reported by Rus Alvarez and Guerrier (1994) in callus cultures of *Lycopersicon.* It thus seems that the elevated proline content discerned in salt-stressed plants could be a reflection of the NaCl-induced inhibition of proline oxidase activity as well.

NaCl was found to be highly inhibitory to amylase activity, as the activity of the enzyme decreased significantly by 69% in BR and by 84% in GR_{11} salinized tissues as compared to the control (at the end of the fourth week). The decreased activity of amylase in salt-stressed rice plants has been reported by Prakash and Prathapasenan (1989). However, HP exposure caused a significant stimulation of amylase activity in both cultivars. When grown on medium containing both HP and NaCl, HP-exposed BR callus showed a nearly 56% increase in amylase activity, while GR_{11} callus showed an increase of 59% as compared to its salt control (Table 4).

Table 3
Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on the activity of proline oxidase (units mg^{-1} protein) in rice callus of varieties BR and GR_{11}

Treatments	Variety	Culture duration (weeks)			
		0	2	4	6
Control	BR	5.0 ± 1.0*	14.0 ± 1.3^{c}	11.1 ± 0.8	9.1 ± 0.9
	GR_{11}	7.3 ± 0.8	25.0 ± 1.9	21.2 ± 1.2	18.0 ± 1.3^{d}
HP	BR	5.0 ± 1.0	6.4 ± 0.7^{a}	6.0 ± 0.9^{a}	5.0 ± 0.8^{ab}
	GR_{11}	7.3 ± 0.8	19.0 ± 1.1^{d}	18.4 ± 1.3^{b}	16.9 ± 1.7^{d}
NaCl	BR	14.0 ± 1.3	8.9 ± 1.1	8.0 ± 0.9	5.4 ± 0.6^{b}
	GR_{11}	25.0 ± 1.9	22.0 ± 1.5	17.5 ± 0.9^{b}	15.3 ± 1.4
NaCl in HP-exposed cells	BR	6.4 ± 0.7	7.6 ± 0.9^{b}	6.1 ±1.0a	4.5 ± 0.6^{ab}
	GR_{11}	19.0 ± 1.1	18.2 ± 1.2^{d}	15.2 ± 0.9	12.8 ± 1.5^{c}
NaCl+HP in HP-exposed cells	BR	6.4 ± 0.7	6.8 ± 0.8^{ab}	5.8 ± 0.7^{a}	4.0 ± 0.6^{a}
	GR_{11}	19.0 ± 1.1	15.0 ± 1.1^{c}	12.5 ± 1.2	11.8 ± 1.6^{c}

Values in the same column with the same superscript do not differ significantly ($P < 0.05$) according to the LSD test
*Values indicate ± Standard deviation of three separate measurements

Table 4
Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on the activity of amylase (units mg^{-1} protein) in rice callus of varieties BR and GR_{11}

Treatments	Variety	Culture duration (weeks)			
		0	2	4	6
Control	BR	12.22 ± 1.6*	38.29 ± 2.8	62.98 ± 1.1^{a}	27.96 ± 0.7^{b}
	GR_{11}	11.84 ± 0.6	18.95 ± 1.4	61.69 ± 1.2^{a}	16.10 ± 0.4^{a}
HP	BR	12.22 ± 1.6	61.58 ± 1.8	71.21 ± 0.9	28.52 ± 0.9^{b}
	GR_{11}	11.84 ± 0.6	72.95 ± 1.3	85.28 ± 1.0	19.33 ± 1.3^{a}
NaCl	BR	38.29 ± 2.8	9.83 ± 1.4^{b}	19.36 ± 0.7	14.36 ± 0.7
	GR_{11}	18.95 ± 1.4	7.50 ± 0.9^{a}	10.20 ± 0.8	7.09 ± 0.1
NaCl in HP-exposed cells	BR	61.58 ± 1.8	12.50 ± 1.0	22.63 ± 1.4	17.69± 0.5
	GR_{11}	72.95 ± 1.3	8.26 ± 0.7^{ab}	13.81 ± 0.8	8.62 ± 0.3
NaCl+HP in HP-exposed cells	BR	61.58 ± 1.8	27.60 ± 0.9	30.18 ± 0.9	24.33 ± 1.0
	GR_{11}	72.95 ± 1.3	9.45 ± 0.6^{b}	16.24 ± 0.9	9.95 ± 0.9^{a}

Values in the same column with the same superscript do not differ significantly ($P < 0.05$) according to the LSD test
*Values indicate ± Standard deviation of three separate measurements

The activity of the invertase enzyme was significantly reduced by NaCl in salt-stressed calli of BR (43%) and GR_{11} (65%). Pre-exposure of the cells to HP helped them to maintain more invertase activity. At the end of the fourth week of incubation a significant rise of 29% and 56% in the activity of invertase was observed in HP-adapted calli of BR and GR_{11}, respectively, when grown on medium containing both NaCl and HP (Table 5).

Table 5
Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on the activity of invertase (units mg^{-1} protein) in rice callus of varieties BR and GR_{11}

Treatments	Variety	Culture duration (weeks)			
		0	2	4	6
Control	BR	56.6 ± 0.6*	40.2 ± 0.7	72.4 ± 0.7	59.0 ± 1.1
	GR_{11}	43.8 ± 0.7	30.9 ± 0.4	70.8 ± 1.2	34.0 ± 0.6[a]
HP	BR	56.6 ± 0.6	52.6 ± 0.9	117.9 ± 1.4	73.6 ± 1.0
	GR_{11}	43.8 ± 0.7	39.6 ± 0.5	110.1 ± 1.6	51.0 ± 0.7
NaCl	BR	40.2 ± 0.7	28.3 ± 0.4[a]	41.5 ± 0.7	28.0 ± 0.3
	GR_{11}	30.9 ± 0.4	23.5 ± 0.5	25.1 ± 0.4	15.6 ± 0.4
NaCl in HP-exposed cells	BR	52.6 ± 0.9	33.1 ± 0.4	47.4 ± 0.6	41.9 ± 0.6
	GR_{11}	39.6 ± 0.5	28.6 ± 0.3[a]	31.0 ± 0.3	25.5 ± 0.3
NaCl+HP in HP-exposed cells	BR	52.6 ± 0.9	36.8 ± 0.3	53.4 ± 0.9	43.4 ± 0.5
	GR_{11}	39.6 ± 0.5	29.9 ± 0.3	39.2 ± 0.5	32.8 ± 1.1[a]

Values in the same column with the same superscript do not differ significantly ($P < 0.05$) according to the LSD test
*Values indicate ± Standard deviation of three separate measurements

The cellulase activity followed a pattern similar to that of invertase, showing decreased activity in the presence of NaCl. Here also, the incorporation of HP into a medium containing NaCl helped to enhance the activity of cellulase in HP-pretreated callus, by 63% in BR and by 70% in GR_{11} (Table 6).

Table 6
Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on the activity of cellulase (units mg^{-1} protein) in rice callus of varieties BR and GR_{11}

Treatments	Variety	Culture duration (weeks)			
		0	2	4	6
Control	BR	33.1 ± 0.6*	46.9 ± 1.2	51.6 ± 1.7	49.0 ± 1.5
	GR_{11}	35.8 ± 0.5	40.4 ± 1.2	48.8 ± 1.0	38.1 ± 1.0
HP	BR	33.1 ± 0.6	52.8 ± 0.9	98.4 ± 1.4	85.6 ± 1.1
	GR_{11}	35.8 ± 0.5	45.3 ± 0.8	53.1 ± 0.7	46.2 ± 1.1
NaCl	BR	46.9 ± 1.2	12.1 ± 0.4	21.3 ± 0.6[a]	16.4 ± 0.7
	GR_{11}	40.4 ± 1.2	14.4 ± 0.8	15.8 ± 0.5	9.8 ± 0.6
NaCl in HP-exposed cells	BR	52.8 ± 0.9	24.0 ± 0.7	30.8 ± 1.0	24.3 ± 0.8[a]
	GR_{11}	45.3 ± 0.8	18.2 ± 0.5	20.5 ± 0.5[a]	12.1 ± 0.7
NaCl+HP in HP-exposed cells	BR	52.8 ± 0.9	32.0 ± 0.6	34.7 ± 0.8	30.3 ± 0.9
	GR_{11}	45.3 ± 0.8	21.4 ± 0.6	26.8 ± 0.9	24.7 ± 0.6[a]

Values in the same column with the same superscript do not differ significantly ($P < 0.05$) according to the LSD test
*Values indicate ± Standard deviation of three separate measurements

NaCl has been shown to reduce growth by inhibiting the activity of invertase and amylase (Morris and Arthur, 1985; Prakash and Prathapasenan, 1989). A similar correlation between reduced growth and a low activity of cellulase and pectin lyase was also shown by Prakash and Prathapasenan (1990).

A significant rise in the activity of IAA oxidase was observed in the calli of both rice cultivars grown under NaCl stress (Table 7). At the end of the fourth week, the IAA oxidase activity of BR callus grown on NaCl-containing medium was nearly 1.2 times more than the corresponding control value, while salinized GR_{11} callus exhibited nearly 1.9 times more enzyme activity. However, BR and GR_{11} calli pre-exposed to HP and grown on medium containing NaCl and HP exhibited a decrease of 34.2% and 9.2%, respectively, in the IAA oxidase activity (as compared to the corresponding salt control values at the end of the fourth week). It is well recognised that IAA oxidase regulates growth by limiting the concentration of IAA (Scott, 1984). Thus, it appears that the favourable effect of HP on the level of IAA (as evidenced by better growth) may have been due to a lowering of the activity of IAA oxidase and/or a reduction in the auxin degradation.

It is therefore surmised that the stimulation of growth observed in the present studies might be due to the promoting effect of hydroxyproline on the endogenous levels of auxin and proline and on the activity of hydrolases.

Table 7

Effect of hydroxyproline (HP, 10 mM) and NaCl (200 mM) on the activity of IAA oxidase (units mg^{-1} protein) in rice callus of varieties BR and GR_{11}

Treatments	Variety	Culture duration (weeks)			
		0	2	4	6
Control	BR	8.6 ± 0.6*	9.6 ± 0.8^{ef}	7.2 ± 0.8^{c}	8.1 ± 0.2^{cd}
	GR_{11}	3.5 ± 0.4	5.8 ± 0.1^{a}	3.4 ± 0.1	6.3 ± 0.3^{a}
HP	BR	8.6 ± 0.6	8.9 ± 1.0^{de}	5.7 ± 0.8^{a}	8.7 ± 0.9^{e}
	GR_{11}	3.5 ± 0.4	6.5 ± 1.3^{ab}	4.8 ± 0.7	6.3 ± 0.4^{a}
NaCl	BR	9.6 ± 0.8	10.2 ± 0.8^{f}	8.9 ± 0.9	9.6 ± 0.4
	GR_{11}	5.8 ± 0.1	7.9 ± 0.8^{cd}	6.5 ± 0.4^{bc}	8.9 ± 0.6^{de}
NaCl in HP-exposed cells	BR	8.9 ± 0.1	9.5 ± 0.5^{ef}	7.9 ± 0.5	8.9 ± 0.3^{e}
	GR_{11}	6.5 ± 1.3	7.1 ± 0.4^{bc}	6.3 ± 1.3^{ab}	8.1 ± 0.3^{cd}
NaCl+HP in HP-exposed cells	BR	8.9 ± 0.1	8.9 ± 0.8^{de}	5.9 ± 0.6^{ab}	7.2 ± 0.5^{b}
	GR_{11}	6.5 ± 1.3	6.3 ± 1.3^{ab}	5.9 ± 0.5^{ab}	7.8 ± 0.8^{bc}

Values in the same column with the same superscript do not differ significantly ($P < 0.05$) according to the LSD test

*Values indicate $\pm$ Standard deviation of three separate measurements

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PHENOL-OXIDIZING ISOENZYMES, MALATE DEHYDROGENASE PATTERNS AND ORGANOGENESIS OF *SOLANUM NIGRUM* L. AS AFFECTED BY LIGHT TREATMENTS

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The phenol-oxidizing enzymes, malate dehydrogenase patterns and organogenesis of *Solanum nigrum* were studied under the influence of different light treatments. Full regeneration potential was found at the base of *S. nigrum* cuttings when they were cultured under normal light conditions (16 h daily light at 100 μmol m^{-2} s^{-1}) on B5 medium supplemented with 0.5 mg/l benzylaminopurine (BAP). This potential decreased strongly under dim light (16 h daily light at 20 μmol m^{-2} s^{-1}) and darkness. These conditions delayed the appearance of adventitious buds on the explants, and decreased the number of shoots/explant and shoot growth. Microshoots and internodal segments formed roots on half strength Murashige and Skoog medium (MS) supplemented with 1 mg/l indolebutyric acid (IBA). In general, dim light and dark conditions reduced the percentage of explants showing root formation, the number of roots/explant and root growth. While shoot and root formation were efficiently induced from callus culture by the induction medium under normal light conditions, dim light and darkness retarded their organogenesis completely.

The isoenzyme profile of phenoloxidases (peroxidases and indophenol oxidases) and malate dehydrogenases was determined during root and shoot formation from microshoot cuttings under normal light, dim light and dark conditions. During shoot formation, the number and/or staining intensity of certain isoenzyme bands increased with an increase in light intensity from dark to normal light conditions. During root formation the isoenzyme expression patterns were nearly the same under different light conditions. From the results obtained in this work it can be suggested that 16 h daily light at 100 μmol m^{-2} s^{-1} enhances the expression of the phenol-oxidizing enzyme system and consequently the organogenesis processes in *S. nigrum*.

Key words: *Solanum nigrum*, indolebutyric acid, benzylaminopurine, light, tissue culture, organogenesis, isoenzymes

Introduction

The photoreduction of O_2, which has been termed the Mehler reaction (Mehler, 1951), is mainly considered as a valve to dissipate excess reducing power, which initially increased with increasing light intensity, reaching saturation at a light intensity similar to or even lower than that required for maximum photosynthetic electron transport (Hodgson and Raison, 1989). Therefore, light induces the production of several reactive oxygen species, which results in significant damage to cellular constituents and even cell death, if protective mechanisms via antioxidation fail to detoxify these toxic oxygen

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species (Hodgson and Raison, 1989; Asada, 1994; Bowler et al., 1994; Elstner and Osswald, 1994; Polle, 1995; Low and Merida, 1996; Paolacci et al., 1997).

The antioxidative function of phenolic compounds is well known (Elstner and Osswald, 1994). They undergo an electrodonating reaction towards reactive radicals, creating phenoxy radicals which may be more stable than the oxygen radical and await reduction by available electron donors such as ascorbate (Elstner and Osswald, 1994). In addition, the balance between pro- and antioxidative functions may, in turn, be regulated by phenol-oxidizing enzymes (phenol oxidases and peroxidases). The activation of the malate valve during the scavenging of reactive oxygen species was also reported (Scheibe and Beck, 1994).

The capacity of phenolic compounds to act as auxin synergists during root formation is well known (Hu and Wang, 1984). Phenolic compounds are oxidized by phenol oxidases in cultured tissues, as a result of which the tissues turn brown. Since harmful phenolic oxidation products are formed in certain species under illumination, the reduction of the light intensity during adventitious organ initiation should be beneficial (Hu and Wang, 1984). The regeneration potential of the cultured cells was dependent on both the scavenging potential and the capability to produce H_2O_2 through NADH-POX activity (de Marco and Roubelakis-Angelakis, 1996a).

It could be seen from previous studies that there are several interactions between light, organogenesis, and the regulation of the balance between pro- and antioxidants via phenol-oxidizing enzymes. Therefore, the aim of this work was to study the effect of different light treatments, resulting in the production of different concentrations of H_2O_2, on organogenesis and on the phenol-oxidizing isoenzymes and malate dehydrogenases of *Solanum nigrum*.

Materials and methods

Plant materials and establishment of shoot culture

Seeds of *Solanum nigrum* L. (Binding et al., 1982) were disinfected by dipping in 5% chlorox solution for 5 min, followed by 5 min dipping in 75% ethanol. The seeds were germinated on hormone-free B5 medium (Gamborg et al., 1968). Shoot cuttings of the germinated seeds were cultured on B5 agar medium supplemented with 0.5 mg benzylaminopurine (BAP). After five weeks many shoots were initiated from the base of each section. These shoots were subcultured for short periods (two weeks to obtain high multiplication rates) on the same medium. All shoot cultures were maintained in 16 h daily light (100 μmol m^{-2} s^{-1}) at 25 ± 1°C without a humidity control.

Establishment of regenerative callus

The establishment of regenerative callus was achieved via protoplast culture. An isolation technique was applied as described by Binding and Mordhorst (1984). Apical tips including about three young leaves were collected, sliced into small segments (1–3 mm) and incubated in enzyme solution (3% Rohament CP, cellulase and pectinase, Röhm 80029) for 14 h. The protoplasts were passed through a 40 μm pore steel sieve to remove undigested cell clumps, and then centrifuged for 5 min at 100 g to sediment the protoplasts as a pellet. The protoplasts were plated as described by Binding et al. (1988). Medium V-KM (Binding and Nehls, 1977)

containing 0.56 mg/l BAP, 1 mg/l NAA and 0.1 mg/l 2,4-D and the organic nutrients of the 8P medium of Kao and Michayluk (1975) was used for protoplast culture. After two weeks the protoplasts were transferred to B5 agar medium (0.8%) containing the hormonal part of V-KM (BTM medium). After a further two weeks the established calli were transferred for the induction of shoot or root formation using the previously described media.

Induction of shoot and root formation, light treatments

The organogenesis experiments were carried out using previously established shoot cultures or calli of *S. nigrum* L. For adventitious root formation, microshoots or stem internode sections as well as calli were cultured on half strength Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with 1 mg/l indolebutyric acid (IBA). Adventitious bud formation was induced from stem internode or microshoot segments or from calli using shoot-induction medium consisting of B5 basal medium, to which 0.5 mg/l BAP was added. A group of sixty stem internodes, microshoots or calli was cultured in three Petri dishes (9 cm width) and considered as a replicate for each light treatment. The first group was subjected to complete darkness. The third was subjected to the general light conditions suitable for shoot growth (16 h daily light at 100 μmol m^{-2} s^{-1}). As an intermediate treatment the second group was subjected to dim light (16 h daily light at 100 μmol m^{-2} s^{-1}). This was achieved by putting the Petri dishes in a small carton covered with white paper. After four weeks, the percentage of explants showing root or shoot formation was determined. The growth of the roots was determined as the length of roots formed on one explant, while the growth of the shoots was determined as mg fresh weight of a single shoot. The number of initiated shoots or roots/explant was also determined.

Isoenzyme analysis

Microshoots cultured under the influence of IBA and BAP and under different light treatments were used for isoenzyme analysis after 8 days. One gram of the plant materials was ground on ice in a mortar in 1 ml of 0.1 M Tris-HCl, pH 7.0 extraction buffer containing 0.2 M cysteine. The homogenate was centrifuged at 15000 × g at 4°C for 15 min. Supernatants were collected for immediate electrophoresis in 7.5% polyacrylamide slab gels. The gels were run at 18 mA for 8 h at 8°C in 0.025 M Tris + 0.192 M glycine buffer (pH 8.9). Three isoenzymes were stained: peroxidase (Brewer 1970, Siegel and Galston, 1976), malate dehydrogenase (NAD dependent) and indophenol oxidase (Brewer, 1970).

Results and discussion

While various auxins and cytokinins induced adventitious organ formation in *S. nigrum*, IBA at a concentration of 1 mg/l and BAP at a concentration of 0.5 mg/l were the most effective auxin and cytokinin for the root and shoot formation of *S. nigrum* at 16 h daily light at 100 μmol m^{-2} s^{-1}. In addition, the regenerative ability of the calli obtained from shoot tip protoplasts was higher than that of calli obtained from mature explants such as stems or leaves (Hassanein, 1998). Internodal and microshoot segments of *S. nigrum* displayed a slight enlargement on the lower side of explants cultured on root or shoot induction medium. This enlargement increased in size and formed unorganized callus or initiated organ primordia depending on the light treatment and phytohormones. In this work 16 h daily light at 100 μmol m^{-2} s^{-1} favoured better shoot organogenesis on cultured stem internode or microshoot cuttings of *S. nigrum* (Fig. 1) in comparison with dim light and dark (Tables 1 and 2).

Microshoot cuttings gave the same results as stem internode segments. The colour of the resulting enlargement at the base of the explants was influenced by the light treatments. Under dark conditions the colour was yellowish-white, but under dim light the colour changed from yellowish-white to light green, especially before the initiation of bud primordia after 11 d. Relatively low light treatment or darkness delayed the appearance of adventitious buds on the explants in comparison to normal light conditions: buds appeared after 7 d under normal light conditions and after 11 days under the other two light treatments. In agreement with these results, Torvegrosa and Bouquet (1996) found that in darkness foliar explants did not show any modification during the first few days, after which the peripheral surface of the petiolar stubs showed a dramatic enlargement. Organogenesis began only when the foliar explants were transferred to the light.

In the presence of light, if the plant tissue contained a relatively high phenol content, which was then oxidized by phenol oxidases, the tissue turned brown or black. In the present work the conditions used were suitable for *S. nigrum* tissue culture, so browning was not detected. *S. nigrum* only turned brown at the cut end of the explant if it was subjected to relatively high salinity stress (unpublished data). Lindfors et al. (1990) found that the onset of tissue browning, before the concentration of phenolic compounds became high, did not cause a loss of vitality but, led to vigorous growth and metabolism.

On the other hand, a reduction in the light intensity retarded shoot formation and decreased the number of shoots formed and their growth on internodal or microshoot segments of *S. nigrum* (Table 2). The mild production of phenolic compounds, which may be induced by light, stimulated improvements in the proliferation and regeneration capabilities of *S. nigrum* as well as in diverse plant species (Benson and Roubelakis-Angelakis, 1994). In contrast to these data, Hu and Wang (1984) reported that a reduction in the light intensity during adventitious organ initiation should be beneficial, since the production of harmful phenolic compounds (oxidation products) was avoided. In order to reduce the accumulation of phenolic oxidates, the tissues should be incubated in the dark for several days or weeks (McComb and Newton, 1981; Monaco et al., 1977).

Table 1

Effect of different light treatments on the percentage of root and shoot formation on microshoot and stem internode cuttings and on calli of *S. nigrum*

Parameter	Dark	Dim light	Normal light
Percentage of shoot formation on stem internode or microshoot cuttings	58	68	100
Percentage of root formation on stem internode cuttings	33	25	55
Percentage of shoot formation on microshoot cuttings	75	55	100
Percentage of shoot formation from calli	0	0	60
Percentage of root formation from calli	0	0	52

Table 2
Effect of different light treatments on the root and shoot organogenesis of *S. nigrum*

Parameter	Dark	Dim light	Normal light
Number of shoots/stem internode or microshoot	5*	9*	16
Fresh weight mg/initiated shoots	50*	50*	60
Number of roots/stem internode or microshoot	5*	7*	9
Length of root system cm/stem internode cutting	1.3	0.8*	2.2
Length of root system cm/microshoot cutting	0.6*	0.3*	2.5

* Means significantly different (t-test) from plant cuttings cultured under normal light conditions (16 h daily light: 100 μmol m^{-2} s^{-1}) at $P < 0.05$.

The most interesting results in this work were that darkness decreased the percentage of explants which responded and formed roots under the influence of IBA (Fig. 2 and Table 1). Also, the number of roots/explant and root growth were retarded (Table 2). In addition, while the percentage of root formation on microshoots was 100%, only 55% of internodal stem cuttings were stimulated to form roots under normal light conditions. This indicates that the presence of leaves and shoot tips as a system for the regulation of endogenous hormones and as a receiver for light was very important for the root organogenesis of *S. nigrum*. It is well known that phenolic compounds serve as auxin synergists during root formation (Hu and Wang, 1984). This suggests that it was the light-induced synthesis of phenolic compounds which synergised the role of IBA in the root formation of *S. nigrum*. The *in vitro* root-inducing capacity of phenolics was demonstrated in apple rootstocks (James and Thurbon, 1979). The stimulation of root growth under light conditions may be due to the reduction in the auxin concentration, which may in turn be due to the oxidation of auxin under illumination conditions.

Calli resulting from the continuous cell division of *S. nigrum* protoplasts under suitable culture conditions (normal light) formed roots or shoots on specific induction media (Table 1). Root and shoot formation were completely retarded when the calli were cultured under dim light or darkness. Under these conditions, in four weeks, the compact light-green calli became friable and yellowish-white. In accordance with other reports light is an important factor during the initiation of callus formation and subsequent plant regeneration via organogenesis (Hu and Wang, 1984) or somatic embryogenesis (Mythili et al., 1999).

Isoenzymes are organ-specific; their expression is usually developmentally controlled. A change in the isoenzyme pattern of any particular organ during a particular phase of development was marked by the appearance or disappearance of the isoenzyme form. Such changes in isoenzyme expression suggest that the genes involved in the synthesis of these isoenzymes are differentially activated during development (Chawla, 1991). A comparison between the organogenesis data and the isoenzyme patterns indicated that light stimulated shoot and root organogenesis as well as increasing the number and/or staining intensity of peroxidases (Fig. 3), indophenol oxidases (Fig. 4) and malate dehydrogenases (Fig. 5),

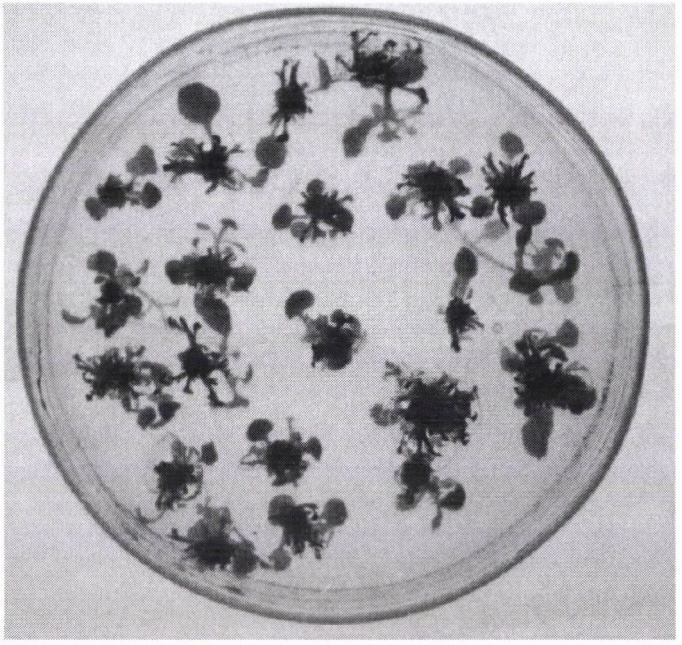

Fig. 1. Photograph of upturned Petri dish (9 cm in diameter) showing shoot formation from the base of *S. nigrum* microshoot cuttings cultured for three weeks on B5 medium supplemented with 0.5 mg/l BAP and subjected to normal light conditions (16 h daily light:100 μmol m^{-2} s^{-1})

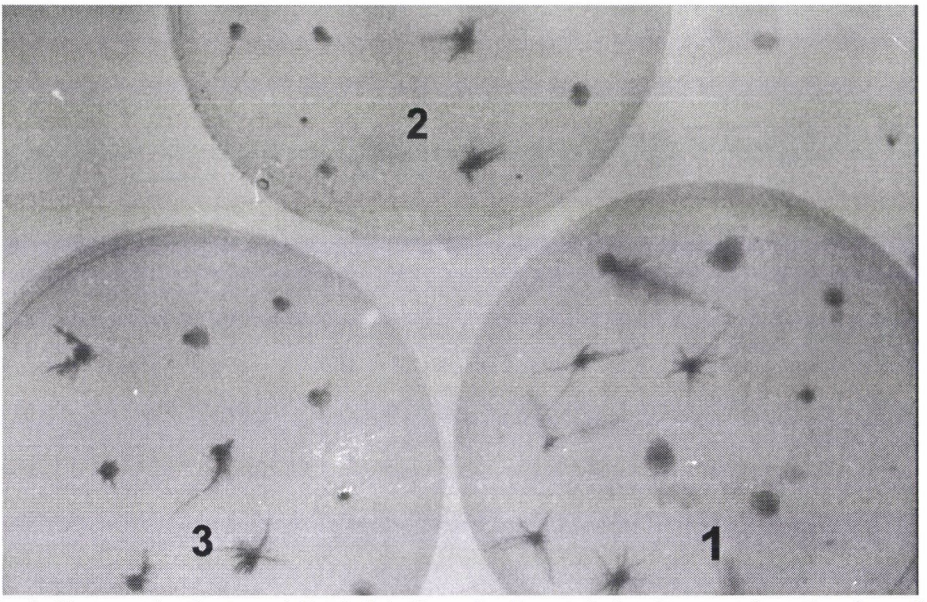

Fig. 2. Photograph of upturned Petri dish (9 cm in diameter) showing root formation from the base of *S. nigrum* stem internode cuttings cultured for two weeks on half strength MS medium supplemented with 1 mg/l IBA. Dish 1: segments subjected to normal light (16 h daily light:100 μmol m^{-2} s^{-1}), dish 2: segments subjected to dim light, and dish 3: segments subjected to darkness

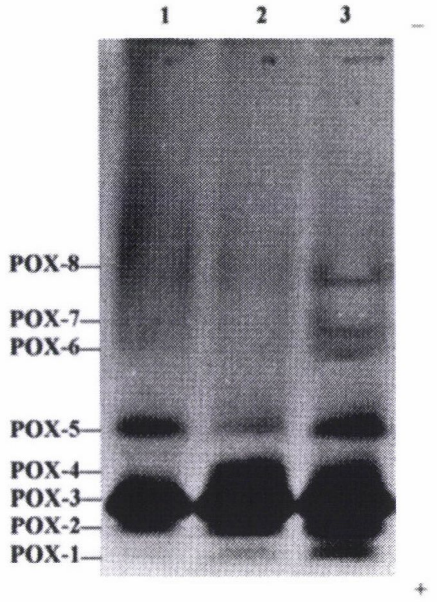

Fig. 3. Native gel electrophoresis of peroxidase (POX) isoenzymes during shoot formation from the base of *S. nigrum* microshoot cuttings cultured on B5 medium supplemented with 0.5 mg/l BAP for 8 d and subjected to different light treatments. Lane 1, darkness; lane 2, dim light (16 hdaily light: 100 μmol $m^{-2} s^{-1}$); and lane 3, normal light (16 h daily light: 100 μmol $m^{-2} s^{-1}$)

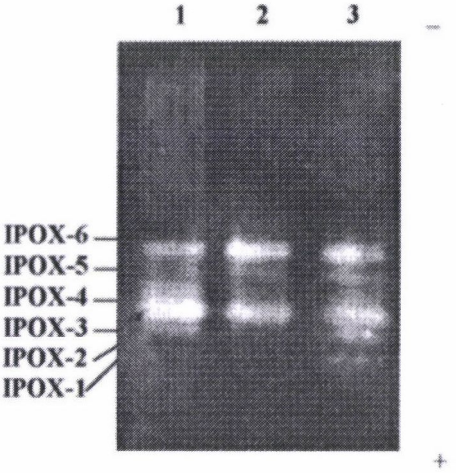

Fig. 4. Native gel electrophoresis of indophenol oxidase (IPOX) isoenzymes during shoot formation from the base of *S. nigrum* microshoot cuttings cultured on B5 medium supplemented with 0.5 mg/l BAP for 8 d and subjected to different light treatments. Lane 1, darkness; lane 2, dim light (16 h daily light: 100 μmol $m^{-2} s^{-1}$); and lane 3, normal light (16 h daily light:100μmol $m^{-2} s^{-1}$)

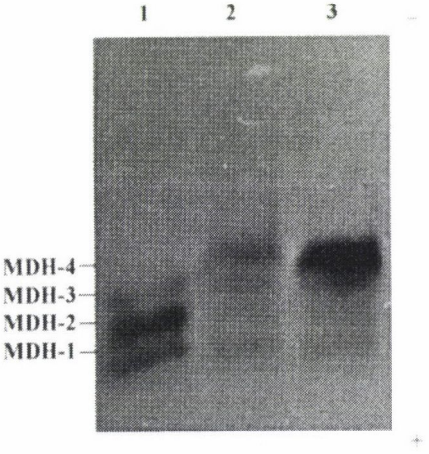

Fig. 5. Native gel electrophoresis of malate dehydrogenase (MDH) isoenzymes during shoot formation from the base of *S. nigrum* microshoot cuttings cultured on B5 medium supplemented with 0.5 mg/l BAP for 8 d and subjected to different light treatments. Lane 1, darkness; lane 2, dim light (16 h daily light: 100 μmol $m^{-2} s^{-1}$); and lane 3, normal light (16 h daily light:100 μmol $m^{-2} s^{-1}$)

especially during shoot formation. It is well known that light enhances the Mehler reaction, which at a low rate (1% of maximum electron flux) would result in the accumulation of a toxic concentration of H_2O_2 (Asada, 1994; Polle, 1995). In general, the rate of the Mehler reaction in isolated thylakoids or chloroplasts was some 2 to 20% of the non-cyclic electron flux (Robinson, 1988). An increase in the staining intensity and the number of isoenzyme forms of peroxidase, indophenol oxidase and malate dehydrogenase, which represents an increase in enzyme activity (Maliga et al., 1978; Khavkin and Zabrodina, 1994; Hassanein, 1998) under light conditions may prevent the accumulation of a toxic concentration of H_2O_2 and consequently enhance the organogenesis of *S. nigrum*. The regeneration potential of the cultured cells was dependent on both scavenging potential and the ability to produce H_2O_2 through NADH-POX activity (De Marco and Roubelakis-Angelakis, 1996a). Phenol oxidases use phenolic compounds to detoxify reactive oxygen species (Elstner and Osswald, 1994). These results are in agreement with those of Benson and Roubelakis-Angelakis (1994), who reported that antioxidants stimulated an improvement in proliferation and regeneration ability in diverse plant species. A parallel relation between the synthesis of phenolic compounds and phenol oxidases was also reported (Nicholson and Hammerschmidt, 1992). Malate dehydrogenase plays a role in scavenging reactive oxygen species via the activation of the malate valve (Polle, 1995). Therefore, new isoenzyme forms of peroxidases [POX-7 and POX-8 (Fig. 3, lane 3), IPOX-1 (Fig. 4, lane 3)] and malate dehydrogenases [MDH-4 (Fig. 5, lane 3)] appeared, especially when the *S. nigrum* explants were induced to form shoots in the presence of light. During protoplast regeneration, a parallel enhancement in cell wall-bound malate dehydrogenase and NADH-POX activities, and the complete rearrangement of the POX isoforms were reported, leading to cell wall reconstitution and the division of cultured protoplasts (De Marco and Roubelakis-Angelakis 1996a; b; Simins et al., 1993). Peroxidases have a role in several physiological processes of plants, including auxin catabolism (Lagrimini et al., 1987). In addition, malate dehydrogenase has been proposed as a component of an apoplastic system that may generate the H_2O_2 required for peroxidase-mediated lignification and the formation of diferuloyl and isodityrosine linkages in the cell wall (Fry, 1986). All these factors may explain the role of light during the shoot formation process. Under light conditions, the increase in root formation and growth on half strength MS medium containing 1 mg/l IBA only led to a slight increase in the staining intensity of a few peroxidase bands.

From this work and other previous studies it can be concluded that light stimulates the scavenging potential of plant cells via an increase in the synthesis of phenolic compounds and phenol oxidases, which stimulate the regeneration potential of *S. nigrum*.

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SPONTANEOUS VERSUS COLCHICINE-TREATED DIHAPLOID PLANTS IN WHEAT (*TRITICUM AESTIVUM* L.) ANTHER CULTURE

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Among 125 well-developed, regenerated green plants derived from anther cultures of four commercial and two new, promising Egyptian wheat (*Triticum aestivum* L.) cultivars, 26 (20.8%) were spontaneous diploids and 99 (79.2%) haploids, as detected by cytological examination. From the 26 spontaneous diploids, 24 (92.3%) grew normally and reached maturity. Except 3 plants (12.5%), all the spontaneous diploid, mature regenerants were fertile. The remaining 99 (haploid) plants were treated with colchicine for chromosome doubling. A total of 53 (53.5%) of the 99 haploids survived the colchicine treatment, of which 24 (45.3%) grew to maturity. Seventeen (70.8%) of the mature plants were fertile. The different genotypes responded differently to colchicine treatment. The genetic differences appear to affect the sensitivity to colchicine, which is why the treated plants varied in their ability to withstand the treatment, to diploidize and/or to be fertile. The viability of the pollen grains of 34 tested, mature plants (16 spontaneous diploids and 18 colchicine-dihaploids) ranged from 41.3% to 95.9%. The plants with low pollen viability (below 54%) were completely sterile. Plants with spontaneous chromosome doubling had higher pollen viability and fertility, compared to the colchicine-treated ones. The relationship between pollen viability and the fertility of plants obtained *in vitro* is discussed.

Key words: wheat - *Triticum aestivum* L., anther culture, haploids, spontaneous doubling, colchicine treatment, pollen viability, fertility

Introduction

Haploid plants have one set of chromosomes in their cells and, therefore, express all their genetic traits. By colchicine treatment homozygous diploids can be obtained from these plants in a single generation. These attributes make the haploids extremely valuable for genetic studies, plant breeding and for genetic transformation experiments (Chu, 1996). In wheat, two methods are generally available for haploid induction: *in vitro* culturing of anthers, microspores or ovaries and chromosome elimination using *Hordeum bulbosum* or maize. However, anther culture is simpler, more efficient and more cost-effective (Bajaj, 1990).

To be used in wheat breeding programmes, haploid-derived plants have to satisfy certain conditions, such as a 42-chromosome number without abnormal

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meiotic behaviour. Spontaneous chromosome doubling in wheat haploids may occur naturally, but only at low frequencies (Liang et al., 1982; He and Ouyang, 1984; Armstrong et al., 1987; Feng and Ouyang, 1989; Ahmed et al., 1997), although it would be highly desirable, in order to avoid the time-consuming and tedious procedure of artificial doubling. The rarity of unreduced pollen mother cells and the lack of anther somatic tissue development indicate that spontaneous diploids (2n = 6x = 42; Kudirka et al., 1986; Henry and De Buyser, 1990) cannot be of maternal origin. Moreover, no genetic observations suggest an F_2-like segregation in the spontaneous diploid progenies of anther-derived F_1 plants (Ouyang et al., 1973). Diploids, tetraploids and other polyploids may be evolved either by endoreduplications, endomitosis and/or nuclear fusion (Amssa et al., 1980; Kudirka et al., 1986; Henry and De Buyser, 1990). The frequency of spontaneous diploids may differ due to several factors, such as anther culture media (Henry and De Buyser, 1990; Ahmed et al., 1997), culture age and culture temperature (Hu, 1985; Metz et al., 1988; Ahmed et al., 1997), cold pre-treatment (Amssa et al., 1980) and genotype (Ouyang et al., 1994; Gustafson et al., 1995; Ali et al., 1996; Ahmed et al., 1997; Mentewab and Sarrafi, 1997).

Colchicine is the most effective agent for chromosome doubling in wheat (Henry and De Buyser, 1990; Redha et al., 1998). For the chromosome doubling of *in vitro* derived haploids, colchicine can be applied *in vitro* to anthers, microspores, embryos or tillers, and *ex vitro*, by treating the regenerated plants (Henry and De Buyser, 1990; Barnabas et al., 1991; Ouyang et al., 1994; Mentewab and Sarrafi, 1997; Redha et al., 1998). Untreated haploid plants are always sterile and spontaneous diploids are fertile, except those exhibiting chromosomal abnormalities (Hu, 1985). Colchicine-treated haploids are mixoploid: some tillers remain haploid and sterile, while others are more or less perfectly doubled and variably fertile (De Buyser and Henry, 1980; Metz et al., 1988).

The present study addresses the influence of the genotype and chromosome duplication on the survival rate, growth and fertility of regenerated wheat plants.

Materials and methods

Plant materials

A total of 125 regenerated green plants were used, derived from anther cultures of four commercial and two new, promising Egyptian cultivars (Giza 139, Giza 163, Giza 164, Giza 165, Sids 2, Sids 5, respectively) of hexaploid wheat (*Triticum aestivum* L.). The anther culture protocol applied for obtaining these plants was described previously by Ahmed et al. (1996; 1997; 1998). Green regenerated plants were transplanted into pots (Ø15 cm) and placed in a growth chamber at 20°C/15°C day/night with a 16-hour photoperiod. After colchicine treatment on the haploids, all the regenerated plants were moved to the field under natural Egyptian winter conditions until harvesting (1996/1997 season).

Cytological analysis of regenerated plants

Mitotic investigation

Root-tips collected from regenerated plants 1–2 weeks after transplantation were treated with 0.05% aqueous colchicine solution for 3.0–3.5 hours, fixed and stained with 2% (w/v) acetoorcein as reported by Ahmed et al. (1997). The chromosome number of well-divided metaphase cells was recorded. Based on the chromosome number of root-tip cells, the transplanted plants were distinguished as spontaneous doubled haploids (2n=42) or haploids (2n=21).

Chromosome doubling by colchicine treatment

Four- to five-week-old haploid plants were removed from the pots and washed with running tap water. The plants were immersed in 0.05% colchicine solution (50 mg colchicine + 2 ml dimethylsulphoxide, made up to 100 ml with double-distilled water) for 5 hours at 20–23°C in the dark, then removed from the colchicine solution, rinsed with running tap water overnight, repotted and placed in the field. This treatment resulted in the gradual withering and dying of the tillers, but the plants survived due to the formation of new tillers from the base (Linde-Laursen, 1975).

Pollen grain viability and fertility of regenerated mature plants

The 34 regenerated, mature plants (spontaneous and colchicine-treated diploids) were sampled randomly for pollen viability studies. The acetocarmine stain (2%, w/v) was used as a pollen viability test (Sapra and Heyne, 1973). Pollen grains were collected from young spikes showing fully developed anthers between 9:00 and 11:00 a.m., after which a drop of acetocarmine stain was added to the pollen on a microscope slide, and viable and non-viable pollen grains were counted. Certain fertility traits of mature, regenerated plants, i.e. number of spikes/plant, percentage of fertile spikes/plant, number of kernels/spike and number of kernels/plant, were also studied.

Results

Spontaneous polyploidy and colchicine treatment

Among the 125 well-developed, regenerated green plants derived from the anther culture of the Egyptian bread wheat varieties, 26 spontaneous diploid plants could be detected (Table 1). The frequency of spontaneous doubled plants with 42 chromosomes was genotype-dependent, being over 50% in Giza 163 and 0% in Giza 139 and Sids 2. Of these 26 plants, 24 (92.3%) grew and reached maturity. These plants represented four genotypes, i.e. Giza 163, Giza 164, Giza 165 and Sids 5. All the spontaneous hexaploid, mature plants were fertile, except for 3 (12.5%) of the Giza 163 progeny, which were sterile (Table 2).

The remaining 99 haploid plants were treated with colchicine (Tables 1 and 3). A total of 53 (53.5%) of the 99 plants survived the treatment, of which 24 (45.3%) grew normally and reached maturity. Among them, 17 (70.8%) were found to be fertile (Table 3). Chromosome counts could not be done at this developmental stage (where the number of plants and spikes was very low). Therefore, pollen viability and plant fertility were used as indicators of the success of chromosome doubling (Tables 3 and 4).

Table 1
Ploidy status and frequency of transplanted, anther culture-derived plants from six Egyptian bread wheat (*Triticum aestivum* L.) cultivars

Donor cultivar	Transplanted green plants	Spontaneous diploids (2n=42)		Haploids (2n=21)	
	Total No.	No.	%	No.	%
Giza 139	24	0	00.0	24	100.0
Giza 163	31	16	51.6	15	48.4
Giza 164	22	3	13.6	19	86.4
Giza 165	5	1	20.0	4	80.0
Sids 2	12	0	00.0	12	100.0
Sids 5	31	6	19.4	25	80.6
Total	125	26	20.8	99	79.2

Table 2
Fertility and frequency of spontaneous diploid plants derived from anther culture of four Egyptian bread wheat (*Triticum aestivum* L.) cultivars

Donor cultivar	At the age of 4–5 weeks	Spontaneous diploid plants obtained					
		At maturity		Fertile		Sterile	
	Total No.	Total No.	%	No.	% [a]	No.	% [b]
Giza 163	16	14	87.5	11	78.6	3	21.4
Giza 164	3	3	100.0	3	100.0	0	00.0
Giza 165	1	1	100.0	1	100.0	0	00.0
Sids 5	6	6	100.0	6	100.0	0	00.0
Total	26	24	92.3	21	87.5	3	12.5

[a] and [b] number of fertile and sterile plants as a % of the total number of mature plants, respectively

Colchicine-treated plants were obtained from anther calli of all six genotypes studied. However, the regenerated haploids of Giza 165 and Giza 139 proved to be the least sensitive to the colchicine treatment and those of Sids 5 the most sensitive. At the same time, the frequency of colchicine-treated plants that reached maturity was 37.5% in Giza 139, in contrast to 66.7% in Giza 165. All the colchicine-treated mature plants of Giza 163, Giza 165 and Sids 5 were fertile, whereas Giza 164, Giza 139 and Sids 2 produced 25%, 50% and 75% fertile plants, respectively (Table 3).

Pollen grain viability and the fertility of regenerated mature plants

In this study, 125 anther culture-derived plants survived for 4–5 weeks after transplantation. Of these 99 haploids were treated with colchicine. Only 48 plants (24 spontaneous diploid and 24 colchicine-treated dihaploids) grew normally and reached maturity, among which 38 plants (21 spontaneous and 17 colchicine-doubled) proved to be fertile (Tables 2 and 3).

Table 3
Effect of colchicine treatment on haploid plants obtained from anther culture of six Egyptian bread wheat (*Triticum aestivum* L.) cultivars

Donor cultivar	Colchicine-treated haploid plants			Mature colchicine-treated plants					
	Total	Survivors after treatment		Total		Fertile		Sterile	
	No.	No.	%	No.	%[a]	No.	%[b]	No.	%[c]
Giza 139	24	16	66.6	6	37.5	3	50.0	3	50.0
Giza 163	15	8	53.3	4	50.0	4	100.0	0	0.0
Giza 164	19	10	52.6	4	40.0	1	25.0	3	75.0
Giza 165	4	3	75.0	2	66.7	2	100.0	0	0.0
Sids 2	12	7	58.3	4	57.1	3	75.0	1	25.0
Sids 5	25	9	36.0	4	44.4	4	100.0	0	0.0
Total	99	53	53.5	24	45.3	17	70.8	7	29.2

[a] number of mature plants as a % of survivors after colchicine treatment
[b] and [c] number of fertile and sterile plants as a % of the total number of mature plants, respectively

Table 4 shows the results of tests on the pollen grain viability and fertility of 34 regenerated, mature plants derived from anther calli of all six Egyptian wheat cultivars. Pollen grain viability (% of stained, normal-size pollen) ranged from 41.3% (Giza 164, plant No. 2) to 95.9% (Sids 5, plant No. 10). However, small-size pollen (0.0–16.1%) was not stainable (Table 4). Plants with low pollen viability gave a low number of kernels/plant (e.g. plant No. 1 of Sids 5, No. 1 of Giza 164 and No. 11 of Giza 163). Plants with pollen viability lower than 54% were completely sterile (plants Nos 1 and 6 of Giza 139, No. 9 of Giza 163 and No. 2 of Giza 164). On the other hand, the highest number of kernels/plant was obtained from plants with high pollen viability (over 90%: 109 seeds/plant for No. 7 of Giza 163 and 53 seeds/plant for No. 10 of Sids 5).

Plants with spontaneous chromosome doubling had higher pollen viability and fertility than the colchicine-treated dihaploids. In general, in the spontaneous diploid plants 85.6% of the pollen was viable, 100% of the spikes were fertile, 20.7 kernels/spike and 39.4 kernels/plant were formed, while in colchicine-doubled plants these parameters were 67.1%, 60%, 2.4 and 7.1, respectively (Table 4). To compare the efficiency of the two duplication systems on plants derived from a single genotype, colchicine-treated plants of Giza 163 showed an average pollen viability of 73.8% and 9.5 seeds/plant, whereas for spontaneous diploids these values were 81.3% and 38.3, respectively. Consequently, the highest number of seeds/spike was shown by the spontaneously doubled plant No. 10 of Sids 5 (53) and by the colchicine-treated plant No. 2 of Sids 5 (15; Table 4). However, there was a positive correlation between pollen viability and each of spike fertility, kernels/spike and kernels/plant; $r = 0.79$, 0.73 and 0.64, respectively.

Table 4
Pollen grain viability and fertility (number of plant spikes and kernels) of 34 colchicine- and spontaneously doubled, regenerated mature plants derived from anther culture of six Egyptian bread wheat (*Triticum aestivum* L.) cultivars

Donor cultivar	PDM*	Plant	Pollen grains[a]		Plant spikes		Kernel number	
(cv)		code	No.tested	% Stained	Total No.	% Fertile	/Spike	/Plant
Giza 139	C*	1	312	53.5	1	0.0	0	0
		3	278	76.9	2	50.0	1.5	3
		4	434	70.9	3	33.3	1.3	4
		6	267	52.1	1	0.0	0	0
	cv. Mean		*1291*	*63.4*	*1.8*	*27.8*	*1*	*1.8*
Giza 163	C	1	332	74.7	3	66.7	3	9
		2	308	63.3	2	100.0	1.5	3
		3	356	90.2	1	100.0	20	20
		4	274	66.8	4	50.0	1.5	6
		mean	1270	73.8	2.5	72.0	3.8	9.5
	S*	5	432	82.2	1	100.0	13	13
		6	498	74.7	1	100.0	5	5
		7	315	94.9	3	100.0	36.3	109
		8	428	91.8	3	100.0	30.7	92
		9	217	47.5	1	0.0	0	0
		11	400	86.0	1	100.0	1	1
		16	397	91.9	2	100.0	24	48
		mean	2687	81.3	1.7	94.1	22.5	38.3
	cv. Mean		*3957*	*77.6*	*2.1*	*81.0*	*11.4*	*23.9*
Giza 164	C	1	319	68.0	2	50.0	0.5	1
		2	223	41.3	1	0.0	0	0
		mean	542	54.7	1.5	33.3	0.3	0.5
	S	5	527	85.0	4	100.0	14.8	59
		6	346	83.8	1	100.0	7	7
		7	379	85.8	1	100.0	8	8
		mean	1252	84.9	2.0	100.0	12.4	24.7
	cv. Mean		*1794*	*69.8*	*1.8*	*72.2*	*7*	*12.6*
Giza 165	C	1	255	64.7	1	100.0	2	2
		2	364	59.3	3	33.3	0.7	2
		mean	619	62.0	2	50.0	1	2
	S	3	306	88.9	3	100.0	24	72
	cv. Mean		*925*	*75.5*	*2.5*	*80.0*	*14.8*	*37*
Sids 2	C	1	437	82.2	10	70.0	2.5	25
		2	282	68.1	5	80.0	4.2	21
		3	296	69.3	9	44.4	0.4	4
	cv. Mean		*915*	*73.2*	*8.0*	*62.5*	*2.1*	*16.7*
Sids 5	C	1	274	54.7	1	100.0	1	1
		2	313	95.5	2	100.0	15	30
		4	319	75.5	3	100.0	2	6
		mean	906	75.3	2.0	100.0	6.2	12.3
	S	5	210	85.2	1	100.0	6	6
		6	217	92.6	1	100.0	28	28
		7	358	84.6	1	100.0	6	6
		8	436	78.9	1	100.0	19	19
		10	487	95.9	1	100.0	53	53
		Mean	1708	87.4	1	100.0	22.4	22.4
	cv. Mean		*2614*	*81.4*	*1.5*	*100.0*	*11.6*	*17.4*
Mean of colchicine plants		18 plts	5543	67.1	3.0	60.0	2.4	7.1
Mean of spontaneous plants		16 plts	5953	85.6	1.9	100.0	20.7	39.4
Grand Mean		**34 plts**	**11496**	**76.4**	**2.5**	**76.0**	**9.3**	**23.2**

[a] Small-size pollen grains ranged from 0.0 to 16.1%, and were not stainable
*PDM: plant duplication method, C: colchicine, S: spontaneous, plts: plants

Discussion

Spontaneous diploidy and colchicine treatment

Plants regenerated from haploid wheat cultures over several years indicated that about 90–95% of them were euploid with 21 or 42 chromosomes and about 5–10% had abnormal chromosome complements (Kudirka et al., 1986; Metz et al., 1988; Bajaj, 1990; Henry and De Buyser, 1990; Ziauddian and Kasha, 1990; Ahmed et al., 1997). Gustafson et al. (1995) showed that 42% of the plants regenerated from isolated microspore cultures were spontaneously doubled (40–44 chromosomes). It can be concluded from our experiments and from similar studies (Ouyang et al., 1994; Mentewab and Sarrafi, 1997) that spontaneous chromosome doubling is related to genotype. Hu (1985) investigated the root-tip chromosome numbers and fertility of 114 microspore-derived plants and found that 93 plants (81.6%) were spontaneously doubled haploid with a root-tip chromosome number of 2n=6x=42. Among these, 85 plants (74.6%) were completely fertile. Of the 10 partially fertile plants, 2 were checked cytologically and found to be aneuploids. Only 19 regenerated plants (16.7%) were haploids and produced no seed.

In this study, all 24 spontaneous diploid plants were fertile except for 3 (12.5%) derived from Giza 163. Some researchers reported similar observations in other varieties. Armstrong et al. (1987), for example, found that only a few plants were sterile among the regenerated, spontaneous hexaploids with 42 chromosomes. Feng and Ouyang (1989), however, found 46.7% sterile plants in a spontaneous eudiploid population.

Of the 99 colchicine-treated plants in the present study 53 (53.5%) survived the treatment, of which 24 (45.3%) grew normally until maturity and gave 17 (70.8%) fertile plants. In an experiment by De Buyser and Henry (1980), 54.4% of the colchicine-treated wheat haploids produced seed and they reported (Henry and De Buyser, 1990) that 70–90% of the treated plants doubled their chromosome number. Muller et al. (1990) also indicated that after chromosome doubling with colchicine, 87% of all the wheat plants set seeds. The present results are thus in agreement with those obtained by other authors working in this field.

Our results also show that different wheat genotypes vary widely in their response to colchicine. As shown, all the mature, colchicine-treated plants of Giza 163, Giza 165 and Sids 5 were fertile, whereas plants from Giza 164, Giza 139 and Sids 2 produced 25%, 50% and 75% fertile plants, respectively, after colchicine doubling. According to Metz et al. (1988), 98% of colchicine-treated "Centurk" haploids set seed, in contrast to 43% of the similarly treated NB88 haploids.

It appears that the genetic background of the anther donor plants affects the sensitivity of the haploid regenerants to colchicine treatment. Therefore, they

may vary in their ability to withstand the treatment, to diploidize and/or to be fertile. The same conclusion was reached by Lelley and Taira (1979), Tanner (1981), Oettler (1982), Ouyang et al. (1994) and Mentewab and Sarrafi (1997). Nevertheless, Henry and De Buyser (1990) stated that the fertility of the spikes harvested on doubled haploid plants (spontaneous or colchicine-treated) gives a good indication of the chromosomal stability.

Pollen viability and fertility of regenerated mature plants

The regenerated mature, spontaneous diploid and colchicine-treated plants were examined for pollen viability and fertility. The pollen grain viability ranged from 41.3% to 95.9%. Plants having low pollen viability (less than 54%) were completely sterile. By contrast, the highest number of kernels/spike and kernels/plant was obtained from plants with high pollen viability. Spontaneously doubled plants had higher pollen viability and fertility than colchicine-doubled ones. These results suggest that there is a relationship between pollen viability and the fertility of plants obtained *in vitro*. Sapra and Heyne (1973) concluded that male fertility in the triticale lines is probably not an important factor of seed set. However, the percentage of pollen viability in their plants ranged from 80 to 95%, compared with 41 to 95% in our anther-derived plants. Some authors (Badr, 1980; Selim and Hussien, 1983; Sayed-Ahmed, 1995) reported a positive relationship between pollen abortion and the occurrence of chromosomal abnormalities in different plant species.

In this study, colchicine-treated wheat plants had a higher total number of spikes/plant and lower fertility (lower percentage of viable pollen, inferior spike fertility, lower number of kernels/spike and per plant) than spontaneous diploid plants. This result indicates that colchicine treatment may influence the genetic control of plant fertility. However, the genetic stability of advanced, successively selfed generations of wheat plants created by both duplication methods requires further investigation.

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EFFECTS OF SEASONS AND HORMONES ON CROSSABILITY BARRIERS AND *IN VITRO* HYBRID DEVELOPMENT BETWEEN *VIGNA RADIATA* AND *V. UNGUICULATA*

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Interspecific hybrids of mungbean (*Vigna radiata*) with cowpea (*V. unguiculata)* were generated in an otherwise incompatible cross after overcoming crossability barriers using *in vitro* culture techniques. Different pre- and post-fertilization barriers were studied and overcome by the application of hormones. Gibberellic acid (GA) application to the pollinated flower buds was more effective in increasing pollen germination (44%) and pollen tube growth (31%). The inhibition of pollen tube growth in the styles of the crosses was reduced by 15–20% after GA application as compared to their selfed parents. Pod set, pod harvest and flower and pod retention percentages were higher in the kharif (July to October) season as compared to the spring (March to June) season.

Low temperatures of 26 to 30°C with 75 to 85% humidity in the kharif season were more favourable for overcoming crossability barriers than the high temperatures (32 to 36°C) and low humidity (40 to 50%) of the spring season. GA treatment significantly increased the pod harvest in the crosses, and hybrid pod growth could be sustained for 9–10 days making it possible to establish embryo cultures. 10% of the total embryos cultured resulted in successful plantlet formation on MS medium supplemented with 0.4 mg/l naphthalene acetic acid and 1 mg/l benzyl amino purine.

Key words: seasons, hormones, crossability barriers, hybrid, *Vigna*

Introduction

The large variability present in different species of the *Vigna* genus can be utilized for breeding better types. Grain legumes of *Vigna*, such as mungbean (*V. radiata)* and cowpea (*V. unguiculata)*, are widely cultivated in Asia. The improvement of mungbean is one of the major objectives of breeders. Hybrids have been obtained between *V. mungo* and *V. radiata* (Gosal and Bajaj, 1983), *V. pubescens* and *V. unguiculata* (Fatokun and Singh, 1987) and *V. mungo* and *V. unguiculata* (Shrivastava and Chawla, 1993), but interspecific hybrids of *V. unguiculata* and *V. radiata* have not yet been reported. The incompatibility between the *Vigna* species hampers the transfer of genes. The present paper reports the different pre- and post-fertilization crossability barriers and methods for overcoming these barriers, and also discusses *in vitro* culture techniques for producing hybrid plantlets.

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Materials and methods

One cultivar each of mungbean (*V. radiata* cv. K851) and cowpea (*V. unguiculata* cv. IT124) were grown in the year 1996 under normal field conditions during the spring season (March to June) and kharif season (July to October). The average temperature and humidity were 32 to 36°C and 40 to 50% in the spring season and 26 to 30°C and 75 to 85% in the kharif season, respectively. The flowers were emasculated one day before opening and pollinated in the morning of the next day. Crosses were made between mungbean and cowpea including the reciprocal cross. To study the effect of hormones on crossability barriers, three treatments were used. H0 – water as control; H1 – gibberellic acid (GA) 100 mg/l solution; H2 – hormonal solution containing 100 mg/l GA, 25 mg/l naphthalene acetic acid (NAA) and 5 mg/l kinetin (KIN). The hormonal solutions were applied daily at the base of the flower bud and inside the flower bud with the help of a hypodermic syringe needle, from immediately after pollination till the buds were harvested, as reported by Tyagi and Chawla (1994).

Crossability studies

Pre-fertilization crossability characters were studied in the spring season. The different characters studied were pollen fall on the stigma surface 24 h after anthesis, pollen germination, pollen tube growth and pollen tube abnormalities at 10 min, 60 min, 6 h and 24 h after pollination. After different times flower buds were fixed in lactoalcohol (1:2) for 48 h and preserved in 70% alcohol. The pistils were stained with cotton blue solution, destained with 40% acetic acid and mounted in pure lactic acid. For each character the data were recorded on ten samples.

Post-fertilization characters were studied in both the spring and kharif seasons. Pod set 48 h after pollination and pod harvest 20 days after pollination were recorded on a hundred pollinated flowers, though more than 200 crosses were made in each combination. Flower and pod retention was recorded at different time intervals from the 1st to the 12th day after pollination on a hundred pollinated flowers. The value at a particular time interval was calculated from the preceding value.

Culture conditions

The developing pods of the crosses were collected between 9 to 11 days after pollination. The pods were surface sterilized with 20% commercial sodium hypochlorite solution (1% active chlorine) for 5 min, followed by 4–5 washings with sterile distilled water. Immature hybrid embryos were excised from the aborted seeds and cultured on basal MS medium (Murashige and Skoog, 1962) supplemented with various growth regulators and 500 mg/l casein hydrolysate (CH). Two types of MS medium were prepared for embryo culture for the following purposes: i) Direct hybrid plantlet formation: MS medium was supplemented with 0.2 or 0.4 mg/l auxin – either indole acetic acid (IAA) or NAA, and 1 mg/l cytokinin – either benzyl amino purine (BAP) or KIN. ii) Organogenesis, callus induction: MS medium was supplemented with 1.0 or 2.0 mg/l auxin – either 2,4-dichlorophenoxy acetic acid (2,4-D) or IAA, and 0.5 mg/l cytokinin – either BAP or KIN, for callus induction. After callus induction, the calli induced from the embryos were transferred to the medium used for direct plantlet formation, containing a low auxin and high cytokinin ratio. Twenty-five embryos were cultured in each hormone treatment. The cultures were incubated at 25±2°C with a 16/8 h photoperiod and a light intensity of 3000 lux. The data were analysed using a three-factor completely randomized design. Data measured as percentages were subjected to angular transformation before analysis to make them linear with normal distribution.

Results

Pre-fertilization characters

Various pre-fertilization characters showed a significant increase after the application of hormones. The parents showed higher values as compared to the crosses at each hormone level (Fig. 1). The H1 and H2 hormonal applications showed a significant increase over the control treatment. There was no difference

between the H1 and H2 treatments in pollen fall, but significant differences were observed for pollen germination and pollen tube growth in the crosses. Pollen fall and pollen germination were better in crosses with cowpea (IT124) as the female parent, while the pollen tube growth of cowpea was better when it was penetrating the style of mungbean as compared to the reciprocal cross. The H1 hormone treatment was better than the H2 treatment, increasing the pollen fall by 20.3%, pollen germination by 44% and pollen tube growth by 31%.

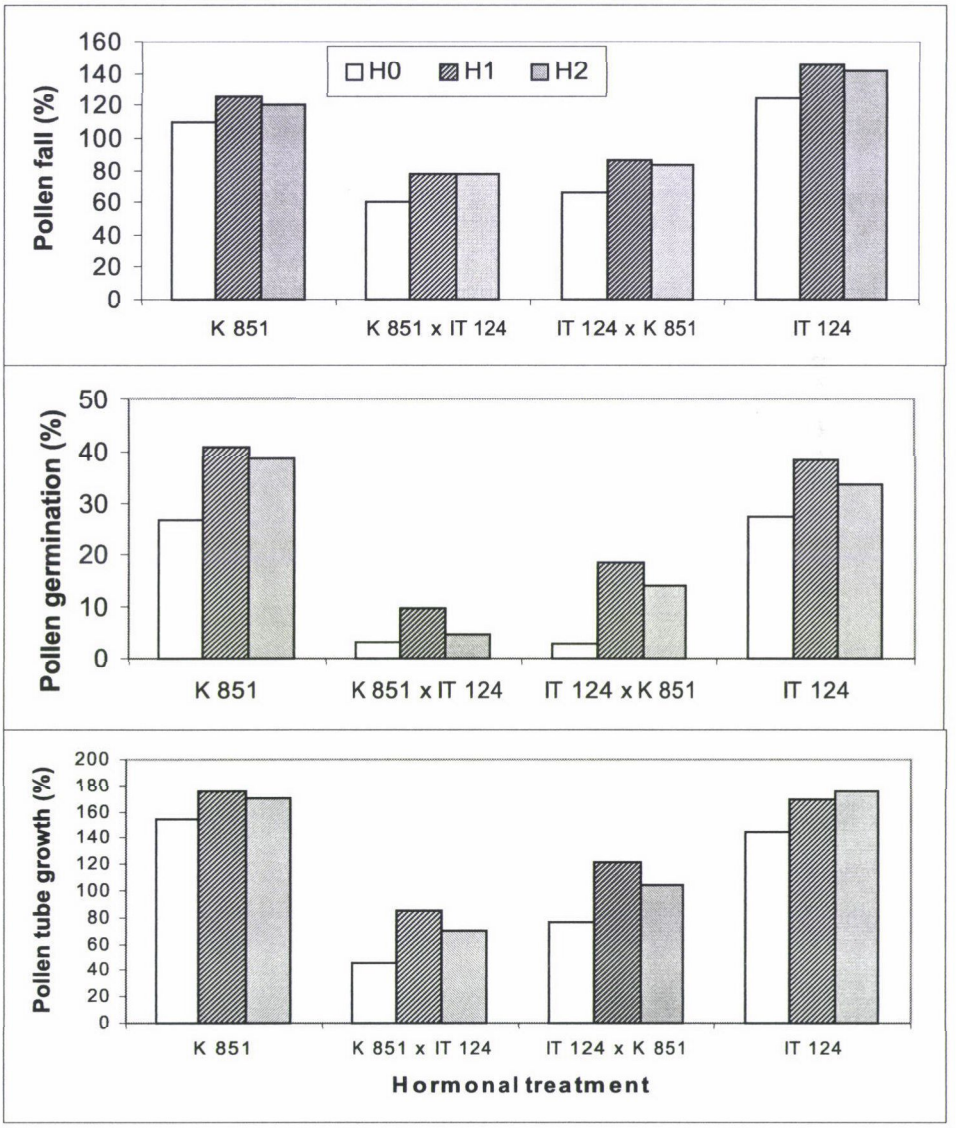

Fig. 1. Effect of hormones on different pre-fertilization characters in *Vigna radiata* × *V. unguiculata* crosses

Pollen tube abnormalities showed considerable variation and both the parents and the crosses showed an increase in the number of abnormalities with the passage of time. Abnormalities were more frequent in the crosses (13.3–14.9%) than in the parents (1.6 to 4%). There was a slight decrease in the percentage of abnormalities after the hormone treatments as compared to the control. The abnormalities observed were pollen tube growth in the wrong direction, the swelling of the pollen tube at the tip, the bursting of the pollen tube at the tip, the coiling of the pollen tube, or two pollen tubes emerging from the same pollen grain.

Post-fertilization characters

All the post-fertilization characters studied showed significant differences in the two seasons, with higher values in the kharif season. The parents showed significantly higher values than the crosses for all the characters and in both the seasons (Table 1). In both the seasons not a single pod could be harvested in the crosses without hormonal application. Over the seasons and crosses it was observed that both the H1 and H2 treatments increased pod set by 7–75% and pod harvest by 28–69%.

The two hormonal treatments studied did not show significant differences amongst themselves. However, the H1 (GA) treatment was better than the H2 treatment. The IT124 × K851 cross, in which IT124 (cowpea) was the female parent, showed a better response for pod set (85%) and pod harvest (69%) after H1 treatment as compared to the reciprocal cross, and the response was better in the kharif season than in the spring season.

Flower and pod retention data values were calculated as the difference (d) for each hormone treatment from the control and were subjected to a two-sample t-test for different crosses as shown in Fig. 2. There were significant differences in the two seasons and pod retention was greater in the kharif season. Hormonal applications led to a substantial increase in percentage flower and pod retention. Pod retention in the IT124 × K851 cross after H1 application was greater as compared to H2 application. This increase was 7–8% and 11% during the kharif and spring seasons, respectively.

Embryo rescue

Hybrid pods of different crosses stopped growing 8–10 days after pollination even after hormone treatment. The developing seeds were shrunken and poorly developed, with the embryos in the heart stage of growth, and generally abortion took place. These embryos were rescued and cultured on different media. In the K851 × IT124 cross a maximum of 20% of the embryos showed germination and only 10% of the total embryos formed plants on the best hormonal combination of 0.4 mg/l NAA and 1.0 mg/l BAP. Small embryos were kept on a medium for callus induction and subsequently a low frequency of shooting was induced. In the reciprocal cross (IT124 × K851) although 26% of the embryos germinated, no plantlets were formed from any of the embryos.

Table 1
Effect of hormonal treatments in different seasons on different post-fertilization characters of *Vigna radiata* and *V. unguiculata* crosses

Parent/Cross	Pod set			Pod harvest		
	Spring	Kharif	Mean	Spring	Kharif	Mean
H0						
K851	75.0	82.0	78.5	75.0	82.0	78.5
	(60.3)	(65.2)	(62.7)	(60.3)	(65.2)	(62.7)
K851 × IT124	0.0	0.0	0.0	0.0	0.0	0.0
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
IT124 × K851	0.0	0.0	0.0	0.0	0.0	0.0
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
IT124	86.0	89.0	87.5	86.0	89.0	87.5
	(69.3)	(72.9)	(71.1)	(69.3)	(72.9)	(71.1)
Mean	40.3	42.7	41.5	40.2	42.7	41.5
	(32.4)	(34.5)	(33.4)	(32.4)	(34.5)	(33.4)
H1						
K851	74.0	84.0	79.0	74.0	84.0	79.0
	(59.5)	(66.8)	(63.2)	(59.5)	(66.8)	(63.2)
K851 × IT124	30.0	60.0	45.0	20.0	28.0	24.0
	(32.8)	(50.8)	(41.8)	(26.1)	(31.6)	(28.9)
IT124 × K851	72.0	85.0	75.5	59.0	69.0	64.0
	(58.1)	(68.6)	(63.4)	(50.2)	(56.3)	(53.2)
IT124	86.0	90.0	88.0	86.0	90.0	88.0
	(69.3)	(73.6)	(71.4)	(69.3)	(73.6)	(71.4)
Mean	65.5	79.7	72.6	59.7	67.7	63.7
	(54.9)	(64.9)	(59.9)	(51.3)	(57.1)	(54.2)
H2						
K851	77.0	33.3	30.0	77.0	83.0	79.1
	(61.7)	(66.0)	(63.8)	(61.7)	(66.0)	(63.8)
K851 × IT124	26.0	56.0	41.0	14.0	26.0	14.6
	(30.2)	(48.5)	(39.4)	(20.5)	(30.4)	(25.4)
IT124 × K851	68.0	82.0	75.0	49.0	66.0	40.5
	(55.7)	(65.2)	(60.4)	(44.4)	(54.4)	(49.4)
IT124	84.0	89.0	86.5	84.0	89.0	87.3
	(66.8)	(73.9)	(70.4)	(66.8)	(73.9)	(70.4)
Mean	63.7	77.5	70.6	56.0	66.0	55.4
	(53.6)	(63.4)	(58.5)	(48.3)	(56.2)	(52.2)
CD (P= 0.01)	$CD^{H}1.97$	$CD^{S}1.60$	$CD^{HS}2.78$	$CD^{H}1.93$	$CD^{S}1.57$	$CD^{HS}2.73$

Data recorded on 100 crosses each as percentage values were converted to angular values as given in parentheses

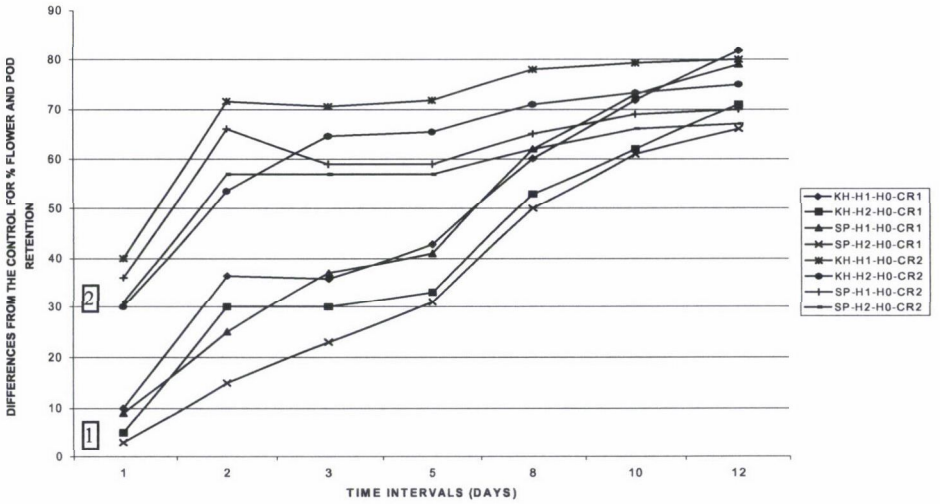

Fig. 2. Pattern of percentage flower and pod retention with different seasons and at different hormones in a *Vigna radiata* ×*V. unguiculata* cross. 1. *V. unguiculata* × *V. radiata.* 2. *V. radiata* × *V. unguiculata*

Discussion

Interspecific hybridization with wild or cultivated species of *Vigna* has been largely unsuccessful, as either the pollen fails to germinate or the union of the gametes does not occur. Before fertilization the interacting partners in pollination are the pollen and the pistil, and physiological and biochemical changes are induced in the ovary before the pollen tube reaches the ovule. Thus, any change in temperature, humidity or hormonal balance in the stylar tissue may change the whole complexion of the interspecific cross and might result in hybrid formation.

Gibberellic acid application led to an increase in pollen characters as well as in post-fertilization characters. The increase in pollen fall might be due to increased stigma receptivity as a result of hormonal application. The adhesion of pollen on the stigma surface involves both impaction and the subsequent formation of attachment bonds (Dumas and Gaude, 1981). On initial contact the adhesive components could be contributed by either or both partners to enhance mutual adhesion (Clark et al., 1979). Pollen germination and pollen tube growth were found to be higher in the parents as compared to the crosses, because species tend to prefer their own pollen. In the crosses where very little pollen germination took place and pollen tube growth was very slow without hormone treatment, hormonal application may have enhanced these two processes so that fertilization could take place. It was seen that GA was more effective than the combination of hormones used. GA increased pollen germination and pollen tube growth in a rye–barley cross (Larter and Chaubey, 1965) and in a *V. mungo* × *V. unguiculata* cross (Shrivastava and Chawla, 1993).

Pollen tube abnormalities were seen mainly in interspecific crosses, the most frequent abnormality being the swelling of the pollen tube at the tip. The occurrence of pollen tube abnormalities points to an incompatible reaction of the pollen tube in the stylar tissue (Sangduen et al., 1983). Ahmad et al. (1988) suggested that crossability barriers might be due to factors operating after fertilization. Post-fertilization characters showed significant differences with respect to the seasons. The higher values recorded in the kharif season as compared to the spring season indicate that the lower temperature of 26 to 30°C and the higher humidity of 75 to 85% during the kharif season helped in overcoming crossability barriers. Pod set, pod harvest and flower and pod retention percentages increased significantly after hormonal application with GA in the crosses, while in the parents the hormones had no effect. The application of GA gave 11% better results than the mixture of GA, NAA and KIN hormones in increasing flower and pod retention. Various workers have reported the use of different hormones and compounds to delay pod abscission (Al-Yasiri and Coyne, 1964; Baker et al., 1975; Gosal and Bajaj, 1983; Chen et al., 1990). Early pod abscission was found to be the main post-fertilization barrier in interspecific crosses involving different species of *Vigna*. With the application of GA, pod growth could continue for 9–10 days after pollination, with the result that the embryos could reach just beyond the heart stage. Seeds in the hybrid pods at that stage were comparable to the growth of 5-day-old seeds in selfed pods. Hybrid embryos with *V. radiata* as the female parent could give rise to plants directly from the embryo or from embryo-derived callus. Chen et al. (1990) and Shrivastava and Chawla (1993) obtained hybrid plantlets through somatic embryogenesis. Thus, crossability barriers can be partially overcome and pod abscission checked for a reasonable time by manipulating the temperature, humidity and hormone treatments so that hybrid embryos can be rescued for raising plantlets.

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MICROCLIMATE MODIFICATION IN SUGAR BEET CANOPY CARRIED OUT BY ROW ORIENTATION

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A field study was conducted at Kehida, Hungary, on silty clay loam soil in 1993–94 to study the influence of different row arrangements on the radiation balance components and plant characteristics of sugar beet. Parallel with microclimate investigations, plant features such as leaf area, stomatal resistance, plant and soil surface temperatures and the yield of sugar beet *(Beta vulgaris)* were measured. Among the components of radiation balance the albedo was determined and radiation penetration into differently oriented rows was modelled. Plants were sown in North-South (N-S) and East-West (E-W) oriented rows. The seasonal weather of the years investigated was similar to each other, but both of them differed from the normal climate: in the growing seasons the weather was hotter and drier than usual. The leaf area of E-W seeded rows was 20% higher than that of the N-S rows. This change, together with the altered radiation properties of different row arrangements, modified other plant parameters and the microclimate as well. Plants seeded in the N-S plot sector had more effective production, accumulating about 7% more dry matter and sugar than plants in the E-W rows.

Key words: modelling relative insolation, row orientation, microclimate, yield

Introduction

Microclimate modifications can be achieved in agriculture by means of the planting direction, irrigation, windbreaks, the use of heaters, irrigation, fans or helicopters to combat spring frosts; and the use of hail or bird nets (Lakso, 1987). Microclimate manipulations in horticultural crops are commonly achieved by canopy design or by shoot positioning or leaf removal. Despite a considerable quantity of research on the sensitivity of fruit trees and grapes to microclimate changes, surprisingly little work has been reported on possible microclimate modifications in arable crops. There are two possible ways of manipulating the microclimate of arable crops through the planting pattern: one is to alter the intrarow plant spacing, and the second is to change the row orientation. The influence of seed spacing on sugar beet production has been reported by Fornstrom and Jackson (1983), Halvorson and Hartman (1984) and Eckhoff et al. (1991), but there is little information about the effect of row exposure on beet plants.

The increase in intercepted radiation in grain legume crops planted north-south was measured by Charles-Edwards and Lawn (1984). However, radiation interception modifications resulting from altered row arrangements were not determined in other plant species, either for soybean (Stützel and Aufhammer, 1991) or for maize (Kasperbauer and Karlen, 1994). The reason for the observed differences between the radiation properties of the investigated plant species can

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be attributed to differences in their leaf movement with respect to the sun. Soybean is a heliotropic plant, while maize is not. Similarly to the radiation properties, a change in row orientation did not influence the morphogenic responses of maize seedlings (Kasperbauer and Karlen, 1994). The yield increase in soybean grown with a N-S row orientation compared to the E-W treatment was consistent with the slightly cooler soil temperatures in the N-S oriented rows (Hunt et al., 1985).

Research to evaluate the effect of exposure on sugar beet production has not been previously reported. The present study was designed to quantify possible alterations in the microclimate and a few plant characteristics resulting from the changed row arrangement. While the radiation penetration into differently oriented rows was modelled, other plant parameters were measured simultaneously with the components of the microclimate on certain selected days in two different growing seasons. Possible explanations for inconsistent plant production responses to altered row alignment were also discussed.

Materials and methods

Field experiments were conducted in the 1993 and 1994 growing seasons on a large commercial sugar beet field cultivated by an agricultural cooperative at Kehida, in Western Hungary (46°45' N; 17°12' E; elevation 105 m) on a silty clay loam soil. On the 5 to 8 selected days studied yearly, from the end of June until the middle of October, the weather was consistent, with clear sky conditions. The wind speed was the only factor that caused differences in the results between the selected days. The profound effect of passing clouds on the temperature level in the field, which is difficult to record, allowed only a limited number of days to be selected as sample days.

The variety *Marika*, a sugar beet cultivar commonly grown in Hungary, was used for the experiment. Seed was sown in conventional rows 0.45 m apart in April and the beet was harvested in October each year. The sites were overseeded and thinned after emergence to the desired 6 plant m^{-2}. One part of the investigated field had its longest dimension in the N-S direction, and the other in the E-W direction. One experimental unit consisted of 100 rows about 200 m in length. Each row orientation treatment was replicated 4 times. The whole of the studied area had a size of roughly 200 m by about 360 m and was surrounded by other fields also planted with sugar beet (Fig. 1). The planting design was completely randomized with row orientation as the treatment. The data were analysed using combined analysis of variance across years.

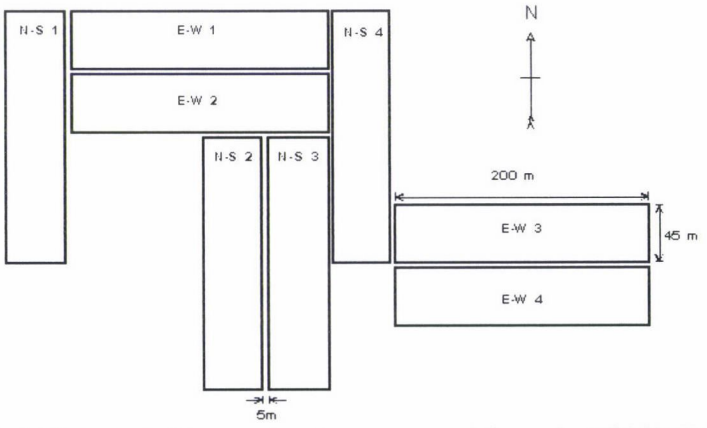

Fig. 1. Placement of the experiment on a commercial sugar beet field in Kehida

Fertilizer applications and pest control were carried out according to the standard agronomic practices for optimum beet production in Hungary.

A mathematical model for evaluating the shading due to plant rows or buildings was developed by Tar and Lóki (1996) and Tar et al. (1998). The model can be used to determine the total insolation period of plant rows on a horizontal plane. The total insolation period consists of times when either one or the other side of the rows is completely insolated by the sun. The model assumes that each day is clear, and there is direct solar radiation only.

Either side of the plant row is wholly insolated when there is no shading by the adjacent rows. The sum of these time periods constitutes the total insolation period. Thus, the shading influence can be calculated using vertically positioned rectangles with different exposure and height, at a given distance from each other, and from those on the ground. Finally, the actual length of the insolation time during clear periods of arbitrary length can be calculated precisely.

The relative insolation period, t_{rel} can be calculated by dividing Δt, the daily period of bilateral (total) insolation, by the length of the day, $\Delta t'$:

$$t_{rel} = \frac{\Delta t}{\Delta t'} \qquad (1)$$

For longer time periods the potential relative insolation period is equal to the sum of the daily values:

$$t_{\Sigma rel} = \frac{\Sigma \Delta t}{\Sigma \Delta t'} \qquad (2)$$

A model LI-60 Steady State Porometer (LI-COR Inc., Lincoln NE, USA) was used to measure the stomatal resistance on days with a completely clear sky and calm weather conditions. The daily change in stomatal resistance was recorded on five to eight days each year after canopy closure, by which time differences in microclimate parameters were manifested. Measurements were taken on the abaxial side of each sunlit leaf of the same age and position, beginning no earlier than 8 a.m. to ensure that the leaves were completely dry.

Two global radiation sensors (LI-200SB) positioned 2 m above the canopy were used to measure the albedo. The air temperature and humidity within and above the plant stands were measured at 3 different levels by commercial sensors connected to a model LI 1000-32 data logger (LI-COR Inc., Lincoln, NE, USA). The lowest psychrometer was positioned close to the soil surface, the next at the top of the canopy, and the third 1 m above the plant stand. Sensors within the plant stands were placed in in-row positions only. Microclimate investigations were carried out on the same completely clear days when the other plant characteristics were also determined. As the greatest change in plant microclimate occurs after canopy closure, the sampling days were chosen when the LAI exceeded 2.5.

Surface temperatures were measured using an infrared thermometer, model Raynger II (Raytek Inc., Santa Cruz, CA) with a 2° field of view, equipped with a band filter of 8 to 14 μm. Before starting the measurements the instrument was thermally equilibrated with the ambient (field) environment for 1 h. The thermometer was hand held 1 m above the plants using an oblique angle of about 30°. The sampling time was 30 s with 5 repetitions in each treatment. The presumed emissivity of the plants was 0.96. Because of the well-known heterogeneity of the soil temperatures determined with traditional mercury thermometers, the soil surface temperature was measured using the same type of IR thermometer. The entire soil surface area between the plant rows was sampled by carrying the thermometer about 0.10 m above the soil for 1 minute hourly between 8 a.m. and 5 p.m. on the selected days. The assumed soil emissivity was 0.90.

At the end of the growing seasons root yields and sucrose contents were evaluated. A single row harvester was used to collect roots from the centre 10.0 m of each replication. The extractable sucrose content was determined by laboratory analysis.

Results and discussion

Weather of the growing seasons

The average air temperatures in the growing seasons were 1.0 and 2.1°C higher in 1993 and 1994, respectively, than the 30-year climatic norm. Each year, this high temperature was associated with a precipitation shortage: the rainfall sum during the two vegetation periods was 23 and 18%, respectively, less than average. The weather of the selected sample days was characterized by 10-day mean deviations from the norm (Fig. 2). Except for the last ten days of June and September in 1993, all the other periods had very hot, dry weather conditions. This means that not only the sample days, but also the previous and subsequent days were characteristic of hot, dry growing seasons.

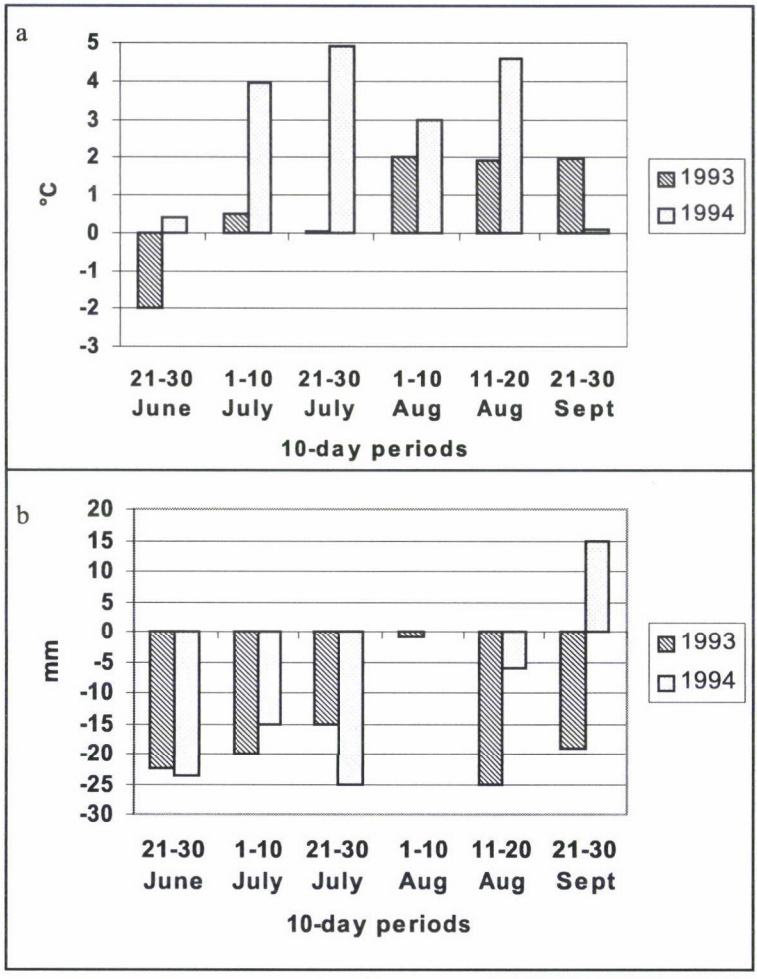

Fig. 2a,b. Deviations from the climatic norm in the temperature (a) and precipitation (b) of sample 10-day periods

Assimilatory surface size

There was no significant difference in either the yearly average LAI or the shape of the yearly variation in assimilatory surface size between the growing seasons of 1993 and 1994. Although the shape of the LAI curves was practically the same in both years, the size of the second peak differed (Fig. 3). There was unusually intensive sprouting at the end of August 1994, which increased the second LAI peak. At the beginning of August 1994, in spite of regular pest control, *Cercospora beticola* and other fungi attacked the plants. After slight leaf defoliation, the beet developed new leaves during the relatively warm autumn. The size of the newly grown leaves was greater in E-W oriented rows than in N-S rows. This sprouting may have made use of sugar accumulated earlier to develop young leaves, and later on this had a negative influence on the root assimilates and final sugar production during 1994.

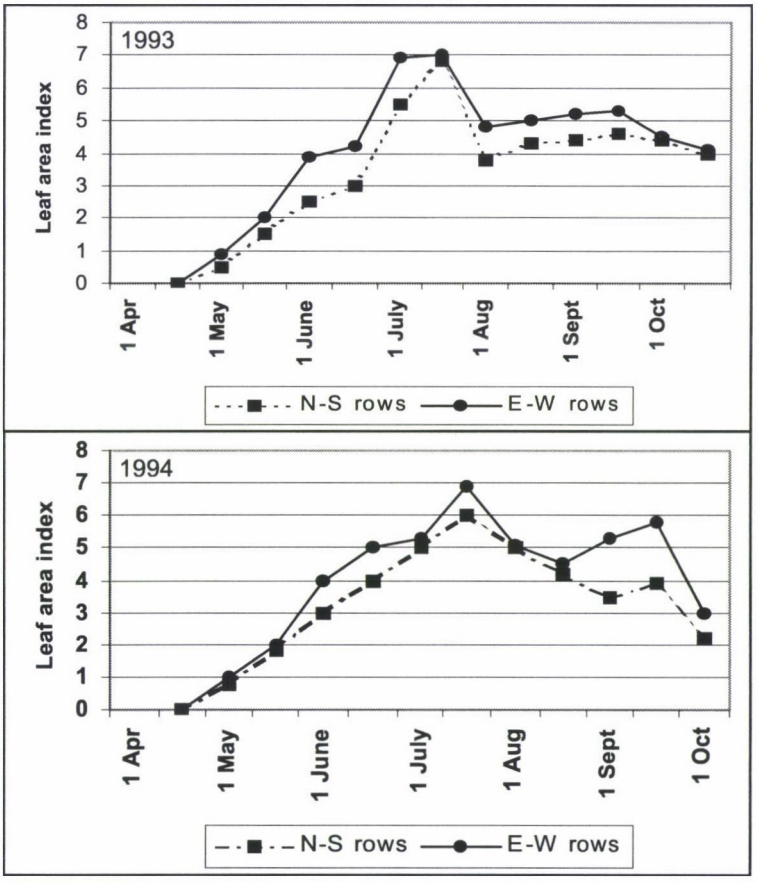

Fig. 3. Seasonal variation of LAI during 1993 and 1994

Independently of the year, sugar beet grown in E-W oriented rows developed a significantly larger assimilatory surface. The LAI of E-W oriented rows was 20.6 and 20% higher in 1993 and 1994, respectively, compared to the assimilatory surface of N-S seeded row treatments.

Radiation properties of sugar beet stands

The results of modelling the total daily insolation time and the relative insolation of two different row treatments are presented in Fig. 4, for the time period when plant height was 0.5 m, and the distance between the rows 0.45 m. During this time, the most important astronomical input of the model, the declination of the sun, decreased from 23° to 8°, but remained positive. In the morning the width of the shadow in E-W seeded rows decreased drastically, reaching 0 very quickly, while later on the sunbeams became parallel with the plant rows. Following the symmetry of radiation, the above variation in the shading of the rows was repeated at sunset as well. Increased radiation at low solar angles in the E-W seeded plots resulted in longer total daily insolation time (about 12 h for 1 July–31 August). As the tendency for bright sunshine duration decreases

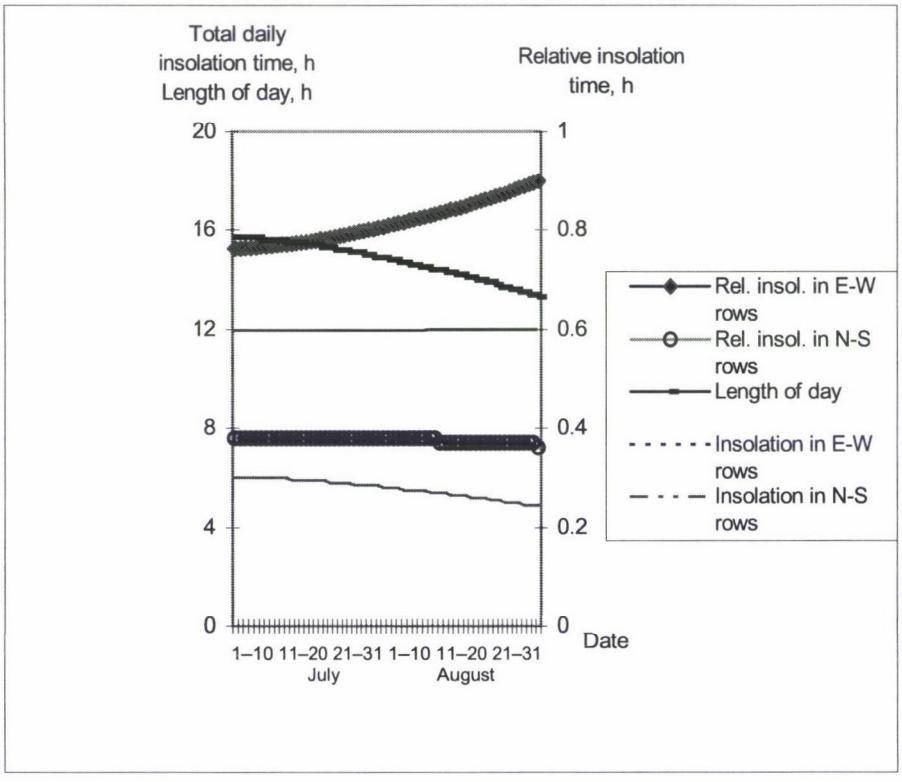

Fig. 4. Modelling the total daily insolation time and relative insolation in two different row arrangements (plant height 0.5 m, spacing 0.45 m, LAI: 2.5)

from July to August, relative insolation was on the increase within the E-W rows. Total daily insolation in the N-S rows decreased parallel with the sunshine duration, and its value was always less than half the total insolation. The reason for this is that the N-S seeded plot sector had only a few hours with undisturbed radiation around the solar noon, when the adjacent rows did not shade each other. The relative insolation in the N-S plot sector remained constant, but only when the plant height (and intrarow plant placement) were equal to the values presented earlier.

Bright sunshine duration for the 2-month period was 913 h. In the E-W and N-S plot sectors the sun shines into the rows for 743 and 345 h, respectively. According to the total insolation time, the 2-month average relative insolation time in the N-S seeded plots was less than half of that determined for E-W seeded rows (0.81 and 0.38).

The seasonal average albedo of the N-S seeded plot sector increased by 8.3 and 7.6% during 1993 and 1994, respectively. There was a tendency for the amount of reflected radiation of N-S seeded rows to increase (to 10–12%) in the second half of the measurements. *Figure 5* shows the seasonal variation in albedo of different row arrangements during 1993. The same results were observed in the following growing season as well. An analysis of the albedo variation indicated that there was 7–8% higher radiation loss in N-S rows compared to the energy lost in E-W rows. The diurnal change in the amount of reflected radiation was independent of the date of investigation.

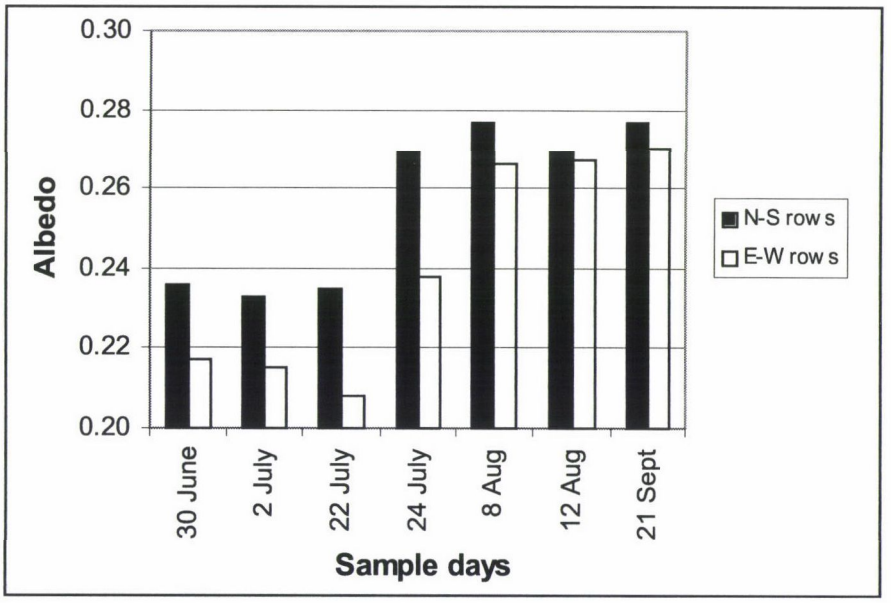

Fig. 5. Daily mean albedos of differently exposed row treatments in 1993

Plant and soil surface temperatures

In calm weather, in spite of the changing radiation penetration in altered row orientations, there was only a slight variation in the daily temperature means of differently placed row treatments. The diurnal patterns of soil temperature curves depended on both solar angles and wind conditions. To discuss changes in the temperature variations of differently placed rows, the sample days were separated into two categories depending on the actual weather, mainly on wind characteristics. The first group contained completely calm days without any significant wind. The second category consisted of windy days, when the average wind speed – measured at 4 m above the canopy – exceeded 3 m sec^{-1}. The effect of row orientation on soil surface temperatures was significant only on completely calm days (Fig. 6a). Due to the increased radiation interception of E-W rows in the morning, increased temperatures were measured. At low solar angles, as there was nothing to stop the sun from shining into the E-W rows, their soil temperature was more than 1.5°C warmer than the soil temperature of N-S oriented treatments. This outcome was in accordance with the earlier findings of Thoefelt et al. (1984). At midday, the above relationship became just the opposite, and the increased temperature appeared in the N-S seeded plant sector. At high solar radiation the temperature difference between the different row arrangements was sometimes as much as 2°C.

On windy days soil temperature was completely dependent on convective heating. A typical pattern of soil temperature as the result of a North wind is presented in Fig. 6b. (This is the prevailing wind direction in Kehida.) The North wind cooled the soil surface of the N-S rows drastically. On calm days, the variance in the daily mean soil temperatures of the investigated two row arrangements was more than 1.5°C, independently of the time of observation. On windy days the wind did not affect the surface temperatures of differently oriented treatments.

The plant temperature patterns of the two growing seasons investigated did not differ significantly. The change in plant temperatures resulting from exposure was the same as that determined for soil surface temperatures. Any treatment difference in plant temperature that originates from the altered row arrangement can only be measured during undisturbed weather conditions. In the morning and evening, the plant temperature of E-W seeded plots was about 1°C higher than that in N-S plots. Similarly to these results, at low solar angle, a moderate increase in canopy temperature in the Western seeded plot sector was reported by Thoefelt et al. (1984). At midday, as the sun shines into N-S rows to a larger extent, this relationship was reversed, and the plant temperature of N-S oriented treatments increased by 1–1.5°C (Fig. 6c). As the duration of periods with low solar angles is at least twice as long as the time period of solar noon, the higher temperatures of E-W seeded plot sectors were generally predominant. There were some exceptions, where the opposite variations in daily mean temperatures resulting from the altered row arrangements balanced each other.

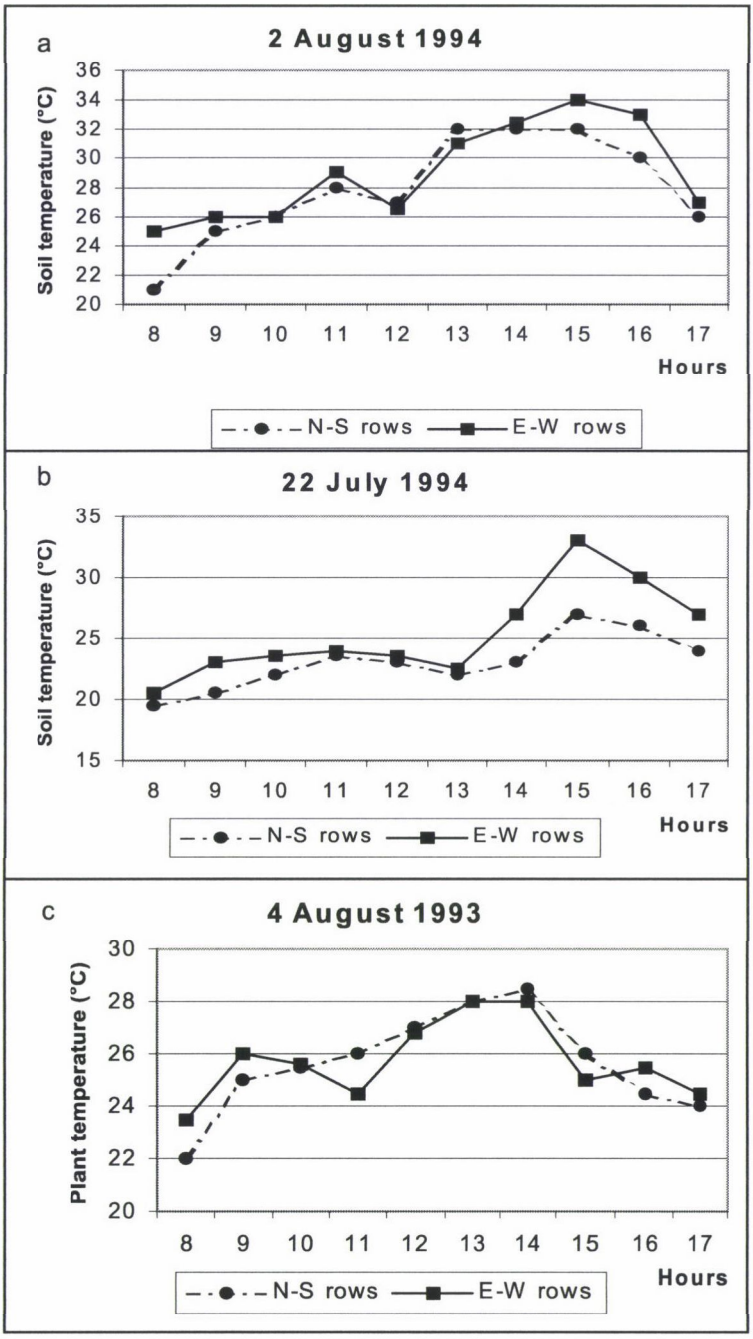

Fig. 6. Typical soil and plant temperatures as affected by row orientation. Soil temperatures are presented for a calm (a) and a windy day (b). Diurnal variation in plant temperature (c) was measured in clear weather

In spite of a 20% precipitation shortage during the summer of 1993, canopy temperatures were not especially high. The calculated yearly mean of 22°C is very close to the 23–25°C optimum temperature interval for sugar beet growth, but a little higher than the optimal temperature (17–21°C) for sugar accumulation determined by Hashemi (1982). A moderate increase in air temperature was observed in 1994, together with some modification in the microclimate: the high air temperatures were associated with increased precipitation amounts during the end of August and September, which influenced the relative humidity of the air around the plants. This increased humidity made the environment favourable for fungus, which attacked and defoliated the plants (see LAI curves). After defoliation the sprouting of new leaves during the warm autumn caused a deterioration in the final yield of the plants in 1994.

Stomatal resistance

Due to the precipitation distribution determined for 1993, the yearly mean resistances were about 15–20% higher than in 1994, depending on the row exposure. The change in the daily mean stomatal resistance of N-S rows exceeded the variation in resistance values determined for the E-W seeded plots (Fig. 7). In August 1993, the resistance measurements were discontinued, because the stomatal resistances reached extremely high (practically infinite) values. Neither the microclimate nor other plant parameters explained this almost complete closure of the stomata. Extremely high stomatal resistances were constant from the middle of August until the end of the 1993 growing season.

In 1993, and with one exception in 1994, the daily mean resistances of the N-S plot sectors were 16.6 and 11.3% higher than the stomatal resistances of the E-W rows, respectively. The only exception occurred in July 1994, when the weather was more moderate than in the previous and later periods of the season. The time when the highest difference in stomatal resistance resulting from changed row direction was recorded coincided with the hottest and driest periods.

Microclimate of plants

The relationship between the air temperature and humidity and the exposure is presented by vertical profiles of these factors measured at the ground and canopy levels, and 1 m above the canopy. As the difference in the microclimate between the two row orientations was the same in the two years investigated, typical variations are presented for selected sample days (Fig. 8a, b).

In the morning and evening, air temperature profiles showed that rows seeded N-S were less than 1°C cooler compared to the air temperature of the E-W seeded plot sector. This is in agreement with the soil temperatures, because at low solar angles the warmer soil of the E-W plots radiates more energy to the

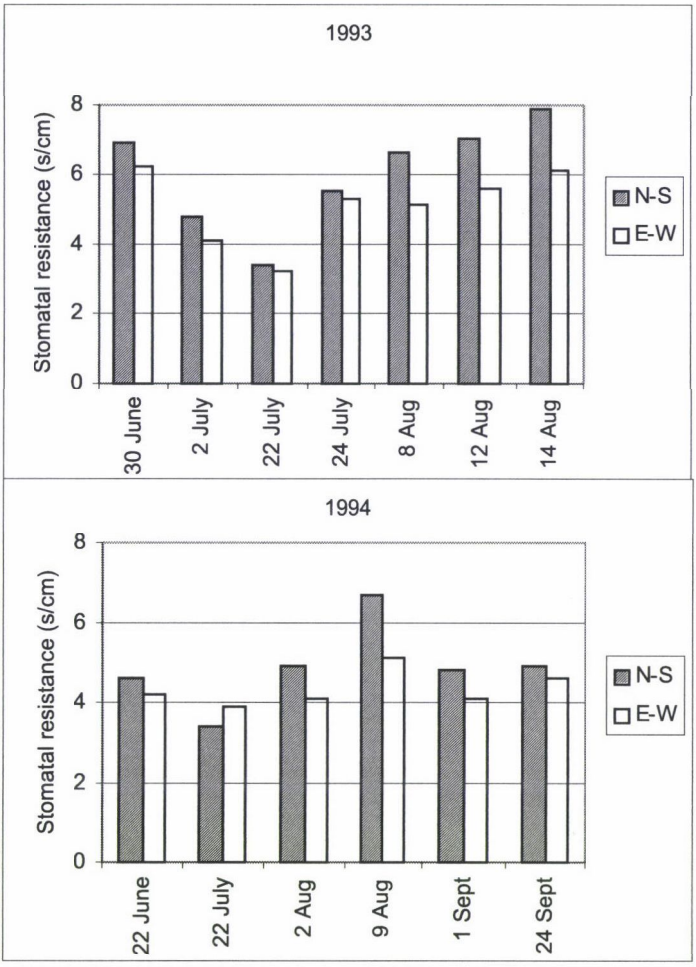

Fig. 7. Yearly variation in stomatal resistance of sugar beet grown in different row arrangements

surrounding air. At midday, on the contrary, the air of N-S seeded rows was warmer than that in the E-W rows. Independently of exposure, the shape of the air temperature curve at midday suggests that most energy was absorbed close to the top of the canopy, where a moderate decrease in air temperature was always observed.

An example of the humidity on calm days with a clear sky is presented in Fig. 8b. In the 2 years of the investigation, the humidity was affected by row orientation in the same way, the plants grown in N-S seeded rows having moister air than sugar beet grown in the E-W plot sector. Unlike the air temperatures, this change was independent of solar angles. It was observed that rainfall influenced the shape of the humidity curves characteristically. Rain changed the source of humidity on sample days just after precipitation. During dry, hot days, from midday, the source of humidity is the canopy itself, and a

moderate increase in the humidity of the top canopy level can be observed. After precipitation the source of humidity all day is the ground, and the highest humidity can be measured close to the soil surface.

There was no significant difference in the components of the microclimate between the different row treatments on windy days. Wind equalized the elements of the microclimate around the plants.

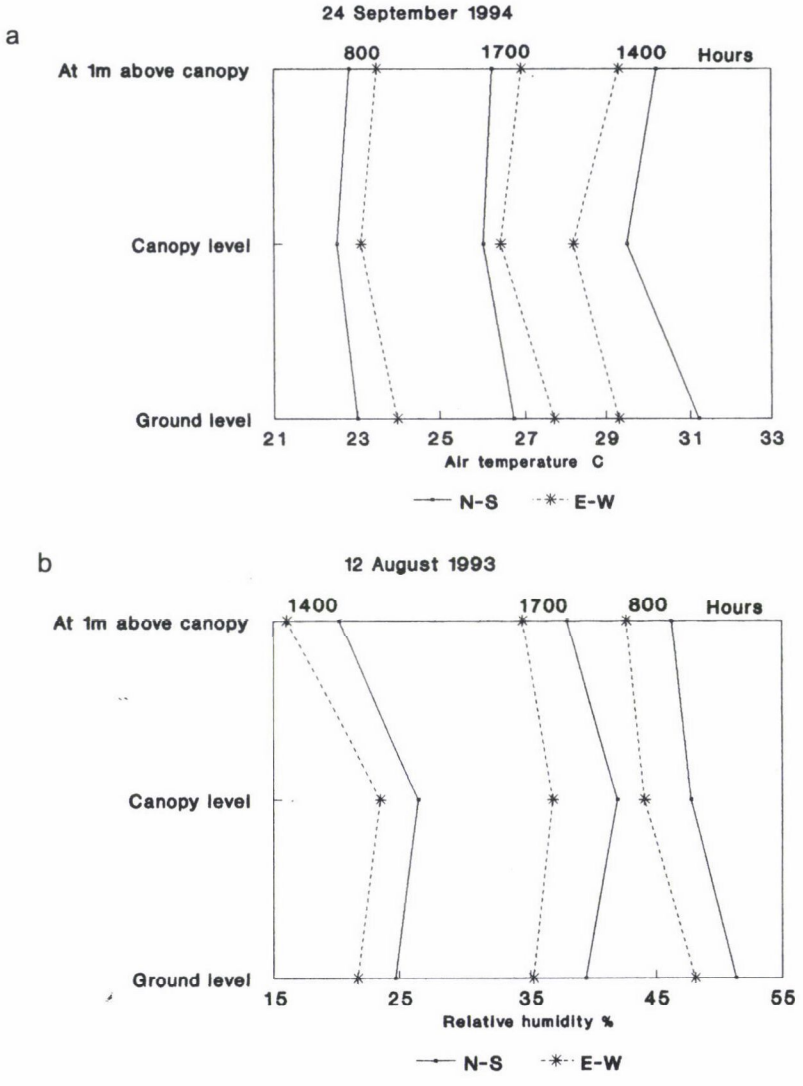

Fig. 8a, b. Representative profiles of air temperatures (a) and relative humidity (b) at different solar angles in N-S vs. E-W oriented rows

Discussion

Summarizing the weather of the years investigated, each growing season was warmer and drier than the climatic norm. The high temperatures were accompanied by less than normal precipitation.

As a result of the direct and indirect influence of the weather in 1994, the sugar yield of sugar beet in each treatment decreased by about one-third compared to 1993 (Table 1). This may have been due to the warm, humid autumn, which had a negative effect on plant health, and later stimulated the plants to develop new leaves. This change probably disturbed the normal sugar accumulation of the roots.

Table 1
Yield components of sugarbeet in differently oriented rows

	Root yield kg m^{-2}		Sucrose content %		Sugar yield kg m^{-2}	
Row orientations	N-S	E-W	N-S	E-W	N-S	E-W
1993	7.9	7.1*	17.5	18.2*	1.39	1.3*
1994	6.4	4.4**	6.3	8.7*	0.41	0.38*

* Difference significant at the 0.05 level
**Difference significant at the 0.001 level

Significant differences in the beet production of the different row orientations were evident in each year. The greater assimilatory surface of plants in the E-W rows increased the sucrose content, but did not lead to a higher root yield compared to that of the N-S row treatments. The sucrose content of the E-W seeded plant sector was 3.9 and 31.1% greater in 1993 and 1994, respectively. The final sugar yield of the E-W rows averaged 6.7 and 7.6% less in 1993 and 1994 than in N-S oriented rows. Other investigators have reported the same kind of relationship for bush bean and soybean (Hunt et al., 1985; Kaul and Kasperbauer, 1988).

The increase in the sugar and root yields of the N-S seeded plant sector was associated with changes in the radiation and water balance components of differently exposed plants. Our radiation model calculated twice as much incoming radiation for the E-W seeded plot sector than for N-S rows. The E-W rows are "open" laterally for the sun at low solar angles. The higher albedo of N-S rows caused a further increase in the energy input of E-W exposed plants. The higher energy source of E-W rows caused an increase in all the temperatures (soil, plant and air) in the morning and evening. As the duration of the time with low solar angles was approximately twice as long as the time period of solar noon, the lower temperatures of the N-S seeded plot sectors promoted a more favourable environment for sugar beet development than those in E-W rows.

Some of the water balance components changed significantly in the different row arrangements. In contrast to the temperatures, modifications in humidity and stomatal resistance were independent of the time of investigation. Both the stomatal resistance and humidity of N-S seeded rows increased compared to the values for the E-W seeded plant sector. The reason for this decreased resistance in E-W rows might have been the increased transpiration resulting from higher radiation penetration into the canopy. Lower air temperatures in the morning and evening were associated with increased relative humidity. As a rule, the higher the humidity, the higher the stomatal resistance. The increase in stomatal resistance of N-S plant rows probably decreased the water loss of the plants. The water saving in N-S rows during hot and dry seasons positively influenced the final root and sugar yield. Additional information is needed on whether the benefit of N-S rows persists under wetter weather conditions.

Sugar beet originates from higher altitudes with a more moderate climate than exists in Hungary. Beet growing areas in Hungary are to be found near the limit of where, in the majority of years, the weather conditions are reasonable for successful beet production. When the weather of the growing season is hotter and drier than normal, the conditions for sugar beet growing deteriorate drastically. During these seasons a change of row exposure could help in providing better beet-growing conditions. Although the increase in yield achieved by changing row orientation was not very high, this surplus was obtained without any extra expenses and in an environmentally friendly way, so it is worth considering the introduction of this technique.

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ADAPTIVE RESPONSES OF *ALHAGI GRAECORUM* UNDER DIFFERENT HABITAT CONDITIONS

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With the aim of specifying how *A. graecorum* adapts itself under different environmental conditions, 60 sites within three different habitats in Egypt were investigated. During one vegetation period, seeds, plant shoots and soil samples were analysed. The correlation between soil factors and morphological characteristics premises the expression of a phenotype-environment interaction or an ecomorphological pattern. Shoot cultures for plants grown under different habitats showed the same phenotype and isoenzyme patterns. Therefore, the variations recorded in plants grown under different habitats may be due to the influence of the ecological conditions.

Organic compounds tended to accumulate within the shoots of *A. graecorum* grown under halic and xeric habitat conditions. This could play a role in the osmoregulation processes, which represent a part of the physiological response of individual plants under these conditions. The Na^+ content of shoots from different habitats was not proportional to the Na^+ content of the external soil solution. Also, the contents of Cl^- and SO_4^{2-} were lower than the contents of Na^+, K^+, Ca^{2+} and Mg^{2+} within these shoots. This leads to the assumption that the plant has a special mechanism that prevents the accumulation of ions in the cells and/or controls their penetration.

Key words: *Alhagi graecorum*, environmental stresses, halic habitat, mesic habitat, xeric habitat, ecomorphological pattern, isoenzymes

Introduction

Alhagi graecorum Boiss is a common frutescent wild plant belonging to the family *Fabaceae* and widely distributed in Egypt. It is recorded as a sub-dominant species in the communities of halic and xeric habitats, but under suitable environmental conditions it flourishes in a mesic habitat and dominates a well-developed community (Kassas, 1952; Zahran, 1972; 1976; El-Khatib, 1994). Under these conditions, it shows great variation in morphology, which is a characteristic feature under natural conditions.

A plant of such wide habitats is of prime ecological interest and can be used for studying many problems concerning the relationship between the plant and its environment. Therefore, the aim of the present work was to elucidate how *A. graecorum* withstands the adverse conditions of the natural environment and to determine, using tissue culture and isoenzyme techniques, whether the observed morphological differences are a genetic feature of the population or an elastic response to the environment.

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Materials and methods

The study area

Sixty sites located in three regions (20 sites in each), namely, the Kharga Oasis (Western Desert), Wadi Bir El-Ain (Eastern Desert) and the Sohag area (Nile Valley) in Egypt, were selected. These regions represent halic, xeric and mesic habitats, respectively. According to Emberger (1951) and Ayyad and Ghabbour (1986), the climate of the studied areas is hyperarid. Rainfall is the main source of surface water all over the Egyptian Deserts, but the groundwater plays a role in the Western Desert, while the River Nile is the main source for surface water in the valley area.

Soil and plant sampling and analysis

During the vegetative period of *A. graecorum*, three replicates of plant and soil were sampled from the 60 selected sites. The soil samples were collected from a depth of 0 to 40 cm underneath individual plants of *A. graecorum* and mixed to form one composite sample per site. The soil samples were air dried and passed through a 2 mm sieve. The soil analysis was performed according to the U.S. Salinity Laboratory Staff (1954). The concentration of different ions was expressed as milli-equivalents per litre (Wikum and Wali, 1974). Plant identification was carried out according to Täckholm (1974) with the nomenclature revised according to Boulos (1995).

Young shoots of *A. graecorum* were collected (three replicates from each site) and immediately stored in an icebox for analysis in the laboratory. Dry plant tissues were used to prepare plant extracts according to Humphries (1956). An atomic absorption spectrophotometer (Parken Elemer M7D) was used to determine the concentration of Na^+ and K^+ in the extracts of both soil and plant (Allen et al., 1974). Titration methods were used to determine the concentrations of Ca^{2+}, Mg^{2+}, Cl^- and SO_4^{2-}. The carbohydrate, protein, total free amino acids and proline contents of the shoots were determined according to Badour (1959), Lowry et al. (1951), Moore and Stein (1948) and Bates et al. (1973), respectively. Morphological characteristics, including variations in spine, stem and root length, were measured and expressed in cm. Leaf area was measured by the disc method according to Watson and Watson (1953) and expressed in m^2 $plant^{-1}$. Succulence was recorded as the difference between fresh and dry weight, and expressed in g $plant^{-1}$. The growth habit of the plants was recorded under the different conditions.

Tissue culture and isoenzyme technique

Seeds of plants grown in different habitats were collected and disinfected by dipping them for one minute in 5% chlorox solution with the addition of two drops of Tween 80, followed by 5 min dipping in 75% ethanol (v/v). The seeds were germinated on B5 medium (Gamborg et al., 1968) supplemented with 2.5 μM benzyl amino purine (BAP). After five weeks, the percentage of seed germination was calculated. Shoot sections with shoot apices (1–1.5 cm) were cut from the seedling and propagated on B5 supplemented with 2.5 μM BAP. The shoots were rooted spontaneously on normal strength MS medium (Murashige and Skoog, 1962) without phytohormones or on MS medium with 1.5% sucrose and 1 μM IBA. All the shoot cultures were maintained in 16 h daily light (100 μ mol m^{-2} s^{-1}) at 25+1°C without a humidity control.

One gram of shoots (grown for three weeks on MS medium without phytohormones) was ground on ice in a mortar using one ml 0.04 M tris-HCl, pH 7.0, containing 0.002 M cysteine. The homogenate was centrifuged at 15,000 rpm at 4°C for 15 min. Native PAGE was performed in a 7.5 polyacrylamide slab gel. Equal volumes of isoenzyme extract were loaded onto the gel wells for electrophoresis. The gels were run at 10 mA per gel for 6 h at 4°C with 0.025M tris-HCl – 0.192 M glycine buffer, pH 8.9. All the isoenzymes were detected according to Brewer (1970).

Statistical analysis

All the data were subjected to analysis of variance using the multiple comparison test to assess significant differences between the mean values of the measurements at the 0.05 and 0.01 probability levels using the Statistical Analysis System (SAS) program (SAS Institute, 1985). Also, the correlation between soil factors and morphological characteristics was measured using the appropriate procedure of the SAS program.

Results

Soil texture is shown in Table 1. The percentage of clay to silt and sand particles in the mesic habitat is significantly higher ($P<0.01$) than in the xeric or halic soils. The mesic habitat soil is significantly different in its moisture (50%) and organic matter contents (4.3%) from the xeric (7%, 0.10%) and halic (18%, 1.4%) habitats (Table 1). The contents of Na^+, SO_4^{2-} and Cl^- in the halic habitat are higher than in the other habitats. The soil reaction tended to be moderately alkaline, with pH ranging between 7.5 and 8.2 in the three habitats. The different types of habitat showed highly significant differences in their contents of total soluble salts (measured as electric conductance).

The morphological variations of *A. graecorum* were represented by spine, stem and root length, leaf area, growth habit and succulence at the three habitats (Table 2). Broad leaves, short fleshy spines and erect stems were the main morphological features of *A. graecorum* growing in the Nile Valley, which showed significant differences ($P<0.05$) from those grown at the other habitats (Table 2). Plants from Wadi Bir El-Ain had long dry spines and small or reduced leaves in comparison with individuals growing in the other habitats. Also, the horizontal growth of the roots was observed, especially in the dry season, where their length reached 24 cm. Under the high soil salinity of Kharga Oasis, *A. graecorum* produced prostrate individuals. The morphological variations of these plants included succulent small leaves and spines. Moreover, the water content of their tissues was significantly higher than that of plants growing under xeric or mesic conditions (Table 2).

Generally, the correlation coefficient of certain soil variables with morphological measurements reveals that, under mesic conditions, spine and root length inversely correlated with the water content of the soil ($r = -0.559$ and -0.569, respectively). There was a clear correlation between spine length and soil moisture content ($r = 0.73$) and between the reduction in leaf area and soil moisture content ($r = 0.66$) under the droughty conditions of the xeric habitat. The high soil salinity of the halic habitat was positively correlated with the succulence of the plants ($r= 0.65$) and the length of the roots ($r = 0.52$).

Tissue culture revealed that there was no difference in the phenotypic response of plants from the three sites when they were propagated under similar conditions. The isoenzyme bands corresponding to peroxidase (POX), indophenol oxidase (IPX), glutamate oxaloacetate transaminase (GOT), esterase (EST) and glutamate dehydrogenase (GDH) activities were visualized, four of which are shown in Figure 1. The same isoenzymes patterns were found for cultured shoots established from plants grown in the different habitats.

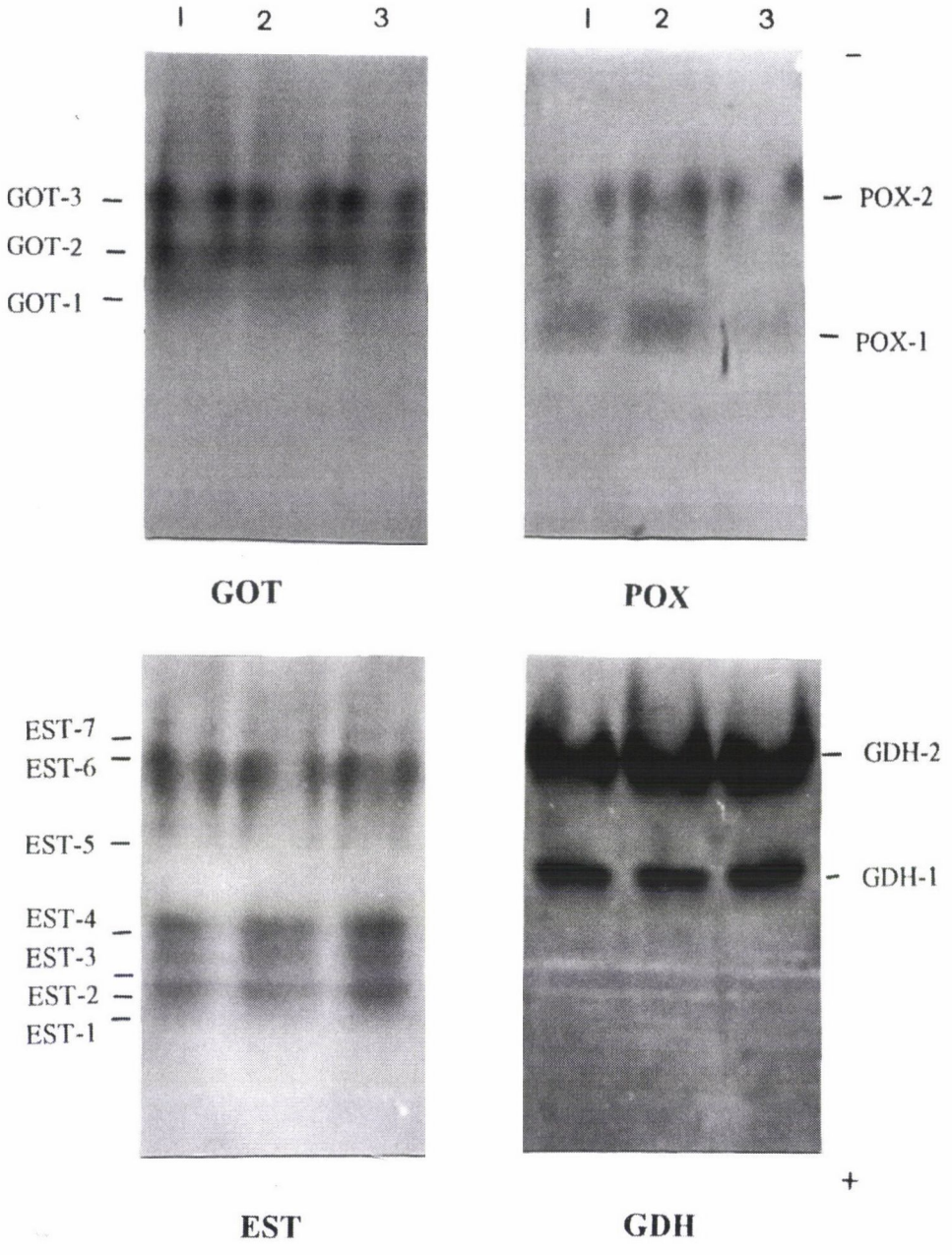

Fig. 1. Isoenzyme patterns of glutamate oxaloacetate transaminase (GOT), peroxidase (POX), glutamate dehydrogenase (GDH) and esterase (EST) present in cultured shoots of *Alhagi graecorum* obtained from mesic (1), halic (2) and xeric (3) habitats. The shoots were grown for two weeks on MS medium without phytohormones

Table 1
Edaphic characteristics (Mean ± SD) of 60 soil samples collected from different *Alhagi graecorum* habitats. All measured parameters were expressed as %, except ions (meq/l) and electric conductivity (μmhos/cm)

Soil characteristics		Habitat types		
		Mesic	Xeric	Halic
Soil texture	Sand*	67 ± 9.5	90 ± 3.9	80 ± 7.0
	Silt*	10 ± 2.3	2 ± 1.4	12 ± 2.6
	Clay*	23 ± 7.9	3 ± 2.4	8 ± 1.0
Moisture content**		50 ± 1.1	7 ± 0.8	18 ± 3.2
Organic matter**		4.3 ± 1.2	0.10 ± 0.05	1.4 ± 0.1
Calcium carbonate**		18 ± 0.4	5 ± 1.2	8 ± 0.3
Ions in 1:5 soil-water extract	Na^{+**}	5.12 ±0.5	9.8 ± 0.1	55.88 ± 4.8
	K^{+**}	7.2 ± 0.3	10.23 ± 0.1	4.14 ± 3.1
	Ca^{2+**}	33.2 ± 0.4	14.05 ± 0.2	5.02 ± 0.3
	Mg^{2+**}	21.5 ± 0.6	9.21 ± 0.4	10.3 ± 2.0
	SO_4^{2-**}	9.8 ± 0.8	10.28 ± 0.1	48.38 ± 2.1
	Cl^{-**}	5.26 ± 0.6	23.21 ± 3.2	30.05 ± 1.3
pH*		7.9 ± 0.2	7.5 ± 0.1	8.2 ± 0.5
Electric conductivity**		264 ± 60	305 ± 45	4900 ± 150

*Difference significant at $P > 0.05$
** Difference significant at $P > 0.01$

Table 2
Means ± SD of some morphological variations in *Alhagi graecorum* under different habitat conditions

Morphological characteristics	Habitat types		
	Mesic	Xeric	Halic
Spine length (cm)**	1.5 ± 0.2	6.0 ± 0.5	3.0 ± 0.3
Leaf area*	5.0 ± 1.2	1.5 ± 0.6	3.0 ± 0.4
Stem length (cm)**	29.0 ± 2.0	18.5 ± 2.1	16.5 ± 1.3
Root length (cm)**	11.5 ± 1.6	24.0 ± 2.0	30.5 ± 0.8
Succulence (g)*	2.9 ± 0.5	3.2 ± 0.2	3.7 ± 0.3
Growth habit	Erect	Erect and prostrate	Erect and prostrate

*Difference significant at $P>0.05$
**Difference significant at $P>0.001$

Table 3 reveals the total carbohydrate contents in plants from the three habitats. Under xeric conditions, the amount of total carbohydrates was 146.30 mg g^{-1} dry wt, while under mesic and halic conditions, the amount of total carbohydrates was lower. The concentration of soluble carbohydrates was not significantly different at the different habitats. The amounts of soluble and insoluble protein in shoots from the halic habitat were significantly higher ($P<0.05$) than in those from other habitats. The total free amino acids content showed a fourfold increase (2.35 mg g^{-1} dry wt) in the xeric habitat and a three-

fold increase (1.80 mg g^{-1} dry wt) in the halic habitat compared to plants from the mesic habitat (0.61 mg g^{-1} dry wt). In the halic habitat, the proline content was nearly five times higher and highly significantly different from that in the mesic habitat (Table 3).

Table 3
Mean values (mg g^{-1} dry weight) of organic and inorganic solutes in *Alhagi graecorum* shoots collected from different habitats

Organic and inorganic solutes	Habitat types		
	Mesic	Xeric	Halic
Total carbohydrate**	80.80	146.30	100.50
Soluble carbohydrate	31.60	32.10	36.10
Soluble protein*	17.25	10.75	29.25
Insoluble protein*	36.25	38.00	62.00
Free amino acids*	0.61	2.35	1.80
Proline*	0.29	0.56	1.41
Na^{+**}	3.10	3.52	4.66
K^{+**}	1.37	1.50	1.88
Ca^{2+**}	12.00	12.00	10.00
Mg^{2+**}	6.00	11.40	6.60
SO_4^{2-}	0.05	0.15	0.21
Cl^{-*}	0.09	0.36	0.57

*Difference significant at $P > 0.05$
**Difference significant at $P > 0.01$

The Na^+ and Mg^{2+} contents of the plant shoots from different habitats exhibited significant differences. The K^+ and Cl^- contents were relatively higher in plant shoots from the halic habitat than in those from the mesic or xeric habitats. In contrast, the Ca^{2+} content was relatively high in plants grown in the mesic and xeric habitats compared to those grown in the halic habitat. Low SO_4^{2-} content in the plant shoots (0.05 mg g^{-1} dry wt) was recorded in the mesic habitat, while the highest Mg^{2+} content was recorded for plants grown in the halic habitat (0.21 mg g^{-1} dry wt), with 0.15 mg g^{-1} dry wt in the mesic habitat.

Discussion

The effect of the physical environment on the distribution and diversity of the vegetation was investigated by El-Khatib (1997) and El-Khatib et al. (1998). They reported that the distribution of plant species depends not only on the genetical and physiological characteristics of the plant, but also on its relationship with the physical environment and its interaction with other species. In arid lands like the study area, the climate affects the equilibrium and maintenance of plant water potential, and its relationship with edaphic factors.

In the present study, the degree of difference between the environmental factors matches well with the results of morphological and physiological characterization. The soil analysis demonstrated the drastic conditions of the xeric and halic soils for the growth of *A. graecorum*. The correlation of edaphic factors with morphological structures emphasizes the intimate link between the phenotype and the environment. The interpretation of the phenotype-environment correlation could be based in part on the convergence of form and function that implies selection by environmental factors and habitat, and represents a first possible mechanism for adaptation of the plant to the environment. In this context, many authors (e.g. Lauder, 1990) elevated this correlation to the status of ecomorphological "rules".

The ecomorphological analyses elucidate the functional relationship between the morphology and ecology of *A. graecorum*, as it is mediated by the behaviour and performance of plant individuals under different habitat conditions. It is clear from the results that the environment itself is complex and impinges upon the morphological characteristics of *A. graecorum* through many processes related to climate and habitat structure. Under the moist regime of the Eastern Desert, the plant individuals have long spines and small or reduced leaves, therefore enabling the plants to grow successfully and resist drought through a reduction in water loss from their transpiring surfaces. In the Kharga Oasis, where the soil contains a high amount of soluble salts, the plant acquires succulent leaves and spines, which seem to be environmentally induced features. Also, the roots are long and penetrate through the saline surface soil to reach the deeper strata (Nubian sandstone, water bearing) where the plants can absorb water. The net effect of this pattern of morphological adaptation is to enable the plant to absorb and transpire the same quantity of water per unit area of plant as mesophytic plants. This plant response to the environment shows the relative importance of ecological adaptation as compared to physiological adaptation. These results are in agreement with the findings of Kassas (1952) and Zahran (1972; 1976), who reported that *A. graecorum* is drought resistant and salt intolerant.

The induction of isoenzyme expression under the influence of different stress conditions has been reported for several plant species (De Jong, 1973; Bergmann, 1987; Peffley et al., 1987; Visedo et al., 1991; Booij, 1995; Alskog and Huss-Danell, 1997). The existence of isoenzyme forms would appear to increase the biochemical versatility of the organism, and to protect it against loss of function occasioned by mutation or environmental stresses (Siegel and Galston, 1967). In contrast, under tissue culture conditions, plants do not need to develop defence strategies such as morphological modifications and metabolic changes. The analysis of isoenzyme patterns is well established in plant physiology studies as a useful genetic and/or biochemical tool to distinguish cultivars from each other (Booij et al., 1995). Controlled and ideal growth conditions can be established under tissue culture conditions. In other words, under such conditions the variations in ecological conditions at the three habitats, that affect the morphology and metabolism of *A. graecorum*, were

excluded. This explains the similarity in the phenotype and isoenzyme patterns of the three plants when they were cultured *in vitro*. This indicates that the phenotypic variations recorded in plants grown in different habitats are due to the ecological conditions.

The second mechanism which helps the plant to withstand adverse conditions is displayed by the cell physiology. The results showed that proline, total free amino acids, protein and carbohydrate accumulated in the plant shoots under both xeric and halic conditions. The differences between the contents of these constituents were significant under the different habitat conditions. In general the amount of proline and total free amino acids in the shoots was very low in comparison with the amounts of soluble carbohydrate and soluble protein. Therefore, it may be argued that carbohydrates and proteins play a more important role in the osmoregulation processes than proline and total amino acids, and represent the first step in this mechanism. In this context, many authors (Joyce et al., 1984; 1992; Batanouny et al., 1985; Daniel and Riyad, 1990; McCue and Hanson, 1990; Hervieu et al., 1994; Hans et al., 1995) have reported that plants may accumulate substances that are normal cell constituents, particularly nitrogen-containing compounds and carbohydrate compounds, during a period of environmental stress. In xeric or halic habitats, the proline content of the shoots increased several times more than in a mesic habitat. However, this increase is not enough to adjust the osmoregulation. This has led to the suggestion that the proline accumulation may merely be a symptom of severe stress (Stewart and Hanson, 1980; Batanouny et al., 1985).

The second possibility of physiological adaptation is the control of ion penetration. The Na^+ content of shoots from different habitats was much the same, and was not proportional to the Na^+ content of the external soil solution. Therefore, the plant has a special mechanism that prevents the accumulation of ions in its cells and/or controls ion penetration, especially Na^+ into the cell. The Cl^- and SO_4^{2-} contents of *A. graecorum* shoots in the three habitats were lower than the contents of Na^+, K^+, Ca^{2+} and Mg^{2+}. In this context, Prat and Fathi-Ettai (1990) suggest that K^+, Ca^{2+}, and perhaps Na^+, were at least partly associated with organic acid anions.

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EVALUATION OF INTERACTION BETWEEN IRRIGATION AND SOIL CULTIVATION IN MAIZE PRODUCTION

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The effect of irrigation and soil cultivation was evaluated by means of variance analysis from the annual and average results obtained between 1989 and 1994 in a long-term experiment set up in the field nursery of Debrecen University of Agricultural Sciences.

The results of variance analysis showed irrigation and soil cultivation to be in close correlation with the yield. The effect of irrigation differed greatly depending on the natural water supplies, but was significant in all the years tested. The interaction between soil cultivation and irrigation was also significant except in 1991, when the yields did not differ significantly from each other thanks to the plentiful rainfall supplies.

Irrigation had the greatest effect when combined with autumn ploughing: the surplus yield averaged over 5 years was 2.87 t/ha. In years with average rainfall the irrigation effect was not significant, but extremely low (0.4 t/ha). In dry years, however, the surplus yield due to irrigation was very large (5.1 t/ha). Judging by the experimental results, minimum tillage cannot be recommended on chernozem soil under irrigated production conditions. There was a significantly smaller surplus yield due to irrigation, which was less efficient than when combined with autumn ploughing. The $LSD_{5\%}$ values showed the effect of irrigation to be significant in the case of spring ploughing, too. Averaged over five years, the surplus yield due to irrigation was favourable. The unfavourable soil moisture status prior to sowing and the almost 50-day period without rainfall in July and August, combined with a daily maximum temperature of over 30°C, greatly reduced the efficiency of irrigation. After spring ploughing the yield was 1.9 t/ha lower than after autumn ploughing. Soil cultivation can only be considered up-to-date and flexible if it is adjusted to the growing site, the soil status, the environmental factors and the farm management conditions.

In the present experiments autumn ploughing was the best soil cultivation method in both irrigated and non-irrigated treatments. The results prove that on chernozem soil, provided rainfall is at least average, autumn ploughing helps in the development of favourable soil status (unhindered root growth, good nutrient availability) and thus substantially increases the yield of maize. Averaged over the experimental years, spring ploughing without irrigation did not differ significantly from minimum tillage. In dry years, however, the water retaining effect of minimum tillage made it significantly more favourable than spring ploughing.

Key words: irrigation, tillage, maize yield

Introduction

In Europe – with the annual 400–600 mm precipitation of the temperate climate – the so-called "classical" irrigation system is practised which, primarily in the growing season, follows the phenological phases of the plants. Bocz (1978) suggests the application of an internationally new, extended irrigation

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system for areas with deeper groundwater levels. In these areas, the upper 200 cm soil layer relies mostly on the atmospherical conditions and in years that are poor in precipitation the 50–160 cm mid-layer of the soil dries out significantly.

Irrigation can ensure a continuous water-supply as well as undisturbed physiological functioning. Parallel with the water shortage, a disorder in physiological functions occurs (Derco, 1979). According to Gulov (1977), in areas that are poor in precipitation, irrigation guarantees a yield. Claassen and Shaw (1970) reported a 53% yield decrease due to drought during tasselling. Water shortage occurring during tasselling and blooming decreases the number of grains, while stress following stamination does the same to their weight, all this resulting in a significant yield decrease (Shaw, 1977). The water needs of a plant significantly decrease as ripening progresses. Then the role of the temperature gains importance (Berényi, 1958; Posza and Stollár, 1983). The experiments of Szász (1973) proved that in Hungary the most decisive yield-regulating factor is the degree of water supply.

According to many authors, the need for irrigation is determined by the water needs of the plant, the method of farming and by the amount of water necessary to maintain favourable soil moisture (Szlovák, 1972; Blanchet, 1973; Cselőtei, 1978; Szőke Molnár, 1977; Petrasovits, 1988; Szalóki, 1988; Szalai, 1989; Jolánkai et al., 1997; Rajkai et al., 1997). The moisture condition of the soil depends primarily on the quantity and dispersion of the precipitation, and secondly on soil conditions, soil cultivation and the position of the groundwater (Várallyay, 1987).

Many methods are known and applied to estimate and determine the lack of precipitation or water deficiency, e.g. Bocz's water supply deficiency, Petrasovits's agrohydropotential, Harnos's drought function, hydrothermic or aridity factors, and the method elaborated by the Irrigation Research Institute to determine potential water deficiency (Szalóki, 1988). The methods can be used well on a given area, by the careful consideration of other factors, to plan irrigation and to determine the necessity of it. According to Szőke-Molnár (1977), irrigation to secure an intensive maize yield will be increasingly important. The right irrigation indicator can only be given in a knowledge of the precipitation and groundwater conditions of the particular field (Balogh, 1978; Posgay, 1983). If precipitation and the available water supply of the soil do not meet the demands of the plant, the deficiency has to be compensated by irrigation (Petrasovits, 1988).

On the basis of the agrometeorological data, in only 25% of the years was there enough precipitation on the Hungarian Plain, as pointed out by Antal et al. (1972). Relying on the findings of maize experiments carried out on two kinds of tillages, Bocz et al. (1983) revealed the reaction of hybrids of different genotypes to the water used for irrigation. While examining several maize hybrids, Nagy (1985; 1987) also found a strong correlation between nutrient supply and irrigation. Irrigation is in close connection with the available soil nutrient supply and nutrient retaining capacity. On the basis of experiments, Debreczeniné (1964) and Debreczeni (1973) stated that on soils with good

nutrient supplies the effect of irrigation or optimal water supply is greater than on soils with lower productivity.

The economicalness of irrigation under given economic conditions is determined by the soil, the nutrient supply and the water utilization efficiency of the plant, in our case: maize. When examining the water requirements of plants over several years, Szlovák et al. (1991) stated that for greater average yield, quantitatively more but relatively less water is required. As was also established by Fekete (1971), irrigation involves the most costs and fixed assets of all the agrotechnical methods and yield-increasing factors.

Examining the impact of irrigation on the environment is an important task. Irrigation is part of the region's complex water supply management and of soil nutrient management, and is an important factor in plant cultivation technologies. Irrigation has an influence on water supply management as well as on plant cultivation, soil being the common basis for it (Várallyay, 1985). The basic requirement is that the applied cultivation technology should not damage the soil structure, should not elicit a long-lasting, airless condition in the soil, should not damage the activity of microorganisms, should not be accompanied by the leaching of N and mineral nutrients, and should not induce secondary alkalization (Fehér, 1954; Stefanovits, 1975; Szabolcs, 1978; Loch and Jászberényi, 1987; Ruzsányi, 1992; Németh, 1992).

On the basis of research, Kovács (1968) pointed out a connection between soil cultivation and the change in soil moisture. The results proved that the effects of soil cultivation on the soil and on the yield cannot be assessed through the results of a single year, but should be evaluated is a soil cultivating system over several years (Sipos, 1958; Nyíri, 1973; Győrffy, 1977; Birkás et al., 1989; Kapocsi, 1984). The packing effects of large, heavy machinery and the increase in the number of operations worsen the physical degradation of the soils (Csizmazia, 1997), which become more obstructed, with poorer water supply management (water drainage, water holding capacity). The need for soil-conserving tillage methods was brought up by Birkás (1987) and Sörös and Soós (1994). When examining the effects of conservation tillage methods it is not sufficient to narrow the assessment down to soil structure. Moisture changes in heat management (circulation), which often shift in an unfavourable direction, should also be examined (Massee, 1982).

According to Herbert (1982), the degradation of soils is the result of several processes. This is confirmed by Stefanovits (1975) and Várallyay (1987) who expanded and redefined the tasks of soil science in agricultural production, and Doran (1982), who emphasized the biological effect of soil cultivation.

The results of the experiment prove that in general, and especially during drought, irrigation increases the yield of maize significantly. In order to achieve a large, stable maize yield, it is essential for farmers to irrigate maize on greater areas. The unusual weather experienced in recent years has focussed special attention on irrigation. Irrigated areas should be expanded wherever possible in maize cultivation.

Materials and methods

At the experimental farm of the Department of Crop Science and Land Use of the Debrecen Agricultural University, the effects of crop cultivation factors on the yield of maize are studied on a chernozem soil with lime deposits. This scientific research is supported by the HAS-DAU Land Use and Research Fund. The polyfactorial long-term experiment makes it possible to evaluate the effects of irrigation and soil cultivation. In the experiments, an irrigated major block and one without irrigation were set up side by side. In the irrigated major block the water demands of the plants were calculated as around 100 mm in 1990, 60 mm in 1991, 170 mm in 1992, 120 mm in 1993 and 110 mm in 1994. The maize hybrids examined were Volga SC, Pannónia SC and Dekalb 524 SC. The size of each tillage block was 4320 m^2. When planning the soil cultivation methods, the goal was to have a difference in the depth of the cultivation as well as in the quality of soil preparation. No ploughing was used in one method, while the depth of general purpose cultivation was 12 cm. Autumn ploughing was done to a depth of 27 cm and spring ploughing, prior to sowing, was done to a depth of 22 cm. The size of each tillage block was 360 m^2 (3 × 360=1080).

The treatments were as follows:

Cultivation	0 = without ploughing
	1 = spring ploughing
	2 = autumn ploughing
Irrigation	0 = without irrigation
	1 = irrigated

Soil characteristics

The soil of the Experimental Farm is chernozem with lime deposits from loess soil. It has moderate supplies of N and P and a high K content (humus content = 2.8–3.0%, total N = 0.14–0.18%, AL-P_2O_5 = 130–200 mg/kg, AL-K_2O = 240–280 mg/kg). The depth of the humus layer is 70–90 cm. The pH value (KCl) is 6.2; the "Arany"-type liquid limit is 43. Microelement deficiency cannot be detected. The groundwater level is between 6–8 m. The minimum water holding capacity (WHC_{min}) of the soil is 27–29 volume %. The water storage capacity of the soil is 275 mm in the 0–100 cm profile and 265 mm in the 100–200 cm profile. The available field capacity (FC) is 157 mm and 150 mm in the 0–100 and 100–200 cm profile, respectively.

Weather characteristics

In Debrecen, over the six years studied, the precipitation was unfavourable (drought) to maize in 1990, 1992 and 1994, while in 1989, 1991 and 1993 the rainfall conditions were average. In order to ensure reliability in the evaluation up-to-date experiment design methods were applied in the planning of the research project, using an improved version of the method developed by Box and Wilson (1951). In the evaluation of the experimental data variance analysis was used, with the disaggregation of the variance components (Sváb, 1981; John, 1971; Winer, 1971). The method of Box and Cox (1964) was used to stabilize the variance of the blocks. For the disaggregation of the variance components the method of "maximum likelihood" was chosen. To evaluate the effects, a mixed, fix-random model was used as suggested by Huzsvai (1994). Within the real replications the hybrids were also considered as replications, since they showed differences over the average of five years. The evaluation was done on an IBM 486 DX computer with the 1988 version of the BMDP Statistical Software.

Results and discussion

The effects of irrigation and cultivation were evaluated annually between 1989 and 1994, as well as over the average of the years with variance analysis, using the results a long-term experiment conducted at the experimental farm of

Debrecen Agricultural University. In 1989, owing primarily to the 379 mm of precipitation that fell in the autumn term, significantly exceeding the 50-year average (243 mm), the yield was exceptional even without irrigation (10.1–11.37 t/ha). The beneficial effect of autumn ploughing was significant: the extra yield was nearly a tonne compared to spring ploughing and 1.36 t/ha compared to the method where no ploughing was used (Table 1). From the plant production aspect tillage achieves its goal if the seedbed is good or at least provides adequate conditions for the germination of maize and for the emergence and development of a uniform crop stand.

In 1990 there was 105 mm less winter precipitation and 168 mm less precipitation in the growing season compared to the average of 50 years. The effect of irrigation in all three cultivation methods was outstanding and significant.

The yield achieved due to irrigation was 4.57 t/ha in the method without ploughing, 5.18 t/ha with spring ploughing and 5.26 t/ha with autumn ploughing. At the same time, the difference between the soil cultivating methods was not significant.

In 1991, the precipitation that fell during the growing season corresponded to the 50- year average (540 mm) and exceeded it in the winter term, so the effect of irrigation was significant but not of great importance in any of the soil cultivating methods. Owing to the favourable water supply, the yield of maize, both in autumn and spring ploughing, showed an average, significant increase of one tonne per hectare compared to the method without ploughing. The correlation between irrigation and soil cultivation was only non-significant in 1991.

Table 1
Effect of irrigation and soil cultivation on the yield. Debrecen, 1989–1994

Tillage	Irrigation	Yield t/ha						Mean
		1989	1990	1991	1992	1993	1994	
Autumn ploughing	Not irrigated	11.37	6.58	10.84	3.99	7.76	4.69	*7.54*
	Irrigated		11.84	11.24	9.25	9.97	9.74	*10.41*
	Mean	*11.37*	*9.27*	*11.04*	*6.62*	*8.87*	*7.22*	*9.05*
Spring ploughing	Not irrigated	10.39	6.68	11.02	2.94	6.54	4.50	*7.01*
	Irrigated		11.86	11.30	9.38	8.26	7.84	*9.73*
	Mean	*10.39*	*9.27*	*11.16*	*6.16*	*7.40*	*6.17*	*8.43*
Without ploughing	Not irrigated	10.01	6.96	9.79	3.63	5.78	4.85	*6.84*
	Irrigated		11.53	10.32	8.55	6.19	9.10	*9.14*
	Mean	*10.01*	*9.25*	*10.06*	*6.09*	*5.99*	*6.98*	*8.06*
$LSD_{5\%}$	Irrigation		0.08	0.20	0.12	0.08	0.07	*0.49*
	Tillage	0.14	ns	0.24	0.14	0.10	0.08	*0.60*
	T×I		0.14	ns	0.20	0.14	0.11	*0.12*

ns = not significant

In 1992, the growing season was dry; there was 188 mm less precipitation compared to the average of 50 years. The situation was aggravated by the fact that precipitation in the winter term was a record low, a mere 92 mm, compared to the average 243 mm recorded over fifty years. Without irrigation, the yield was thus very low, especially in the case of spring ploughing (2.94 t/ha). The method without ploughing and autumn ploughing proved to be significantly better than spring ploughing. The yield achieved with 170 mm irrigation was significantly higher (4.92–6.44 t/ha). Averaged over the non-irrigated and irrigated treatments, the yield achieved with autumn ploughing was significantly greater than with spring ploughing or without ploughing. At the same time, there was no significant difference between the yields achieved with spring ploughing or without ploughing.

The following year, 1993, was more favourable to maize than 1992. In 1993, there was 200 mm of precipitation; this is 50 mm more than in the previous year, but 140 mm less than the fifty-year average. Compared to 1992, the difference in the amount of precipitation did not occur primarily in the growing season, but rather in the winter term: about three and a half times as much rain fell from the harvest of the pre-crop until sowing. There were clear differences in yield. The effect of irrigation was significant. The extra yield achieved by irrigation was 2.22 t/ha after autumn ploughing, 1.72 t/ha after spring ploughing and only 0.41 t/ha where no ploughing was carried out.

The yield after autumn ploughing was more favourable both without irrigation (7.76 t/ha) and in the case of irrigated treatments (9.97 t/ha) compared to no ploughing or spring ploughing. In 1993, both irrigation and soil cultivation were significant, as was their correlation (Table 2). In 1994 the weather was as dry as in 1992: the deviation in the amount of rainfall was only 1 mm. The drought in July and August significantly decreased the effectiveness of plant cultivating factors. The difference was significant primarily after spring ploughing. The effect of irrigation after autumn ploughing and where no ploughing was used was almost identical. Irrigation, soil cultivation and their correlation were all significant (Table 3). According to the results of variance analysis, irrigation and soil cultivation showed a close correlation with the yield. The effect of irrigation showed great differences depending on the natural water supply, but in all the years examined it was significant.

Table 2
Results of variance analysis. Debrecen, 1993

Source of variance	SQ	FG	MQ	F	α-error
Soil cultivation	1195.32834	2	597.66417	1763.28	=0.0000
Irrigation	452.77511	1	452.77511	1335.82	=0.0000
Irrigation × soil cultivation	124.91702	2	62.45851	184.27	=0.0000
Deviation	18.30332	54	0.33895		

Table 3
Results of variance analysis. Debrecen, 1994

Source of variance	SQ	FG	MQ	F	α-error
Soil cultivation	290.31454	2	145.15727	383.16	=0.0000
Irrigation	6372.14520	1	6372.14520	16819.86	=0.0000
Irrigation × soil cultivation	177.83431	2	88.91716	234.70	=0.0000
Deviation	34.096119	54	0.37885		

The interaction between soil cultivation and irrigation was significant as well, except in 1991, when the yields did not differ significantly from each other owing to the more favourable precipitation.

The effect of irrigation was greatest after autumn ploughing; the extra yield over the average of 5 years was 2.87 t/ha. In years with average precipitation, the effect of irrigation is significant but fairly small (0.4 t/ha). At the same time, the extra yield achieved with irrigation in years of drought was significant (5.1 t/ha). According to the results of the experiments, soil cultivation without ploughing is not recommended on irrigated chernozem soil. The extra yield due to irrigation was significantly smaller and its efficiency was worse than after autumn ploughing. On the basis of the $LSD_{5\%}$ values, the effect of irrigation was significant after spring ploughing as well. Over the average of five years, the extra yield from irrigation was favourable, but it became obvious in 1994 that when irrigation is applied, spring ploughing is very risky so it cannot be recommended in practice (Fig. 1). The unfavourable soil moisture conditions

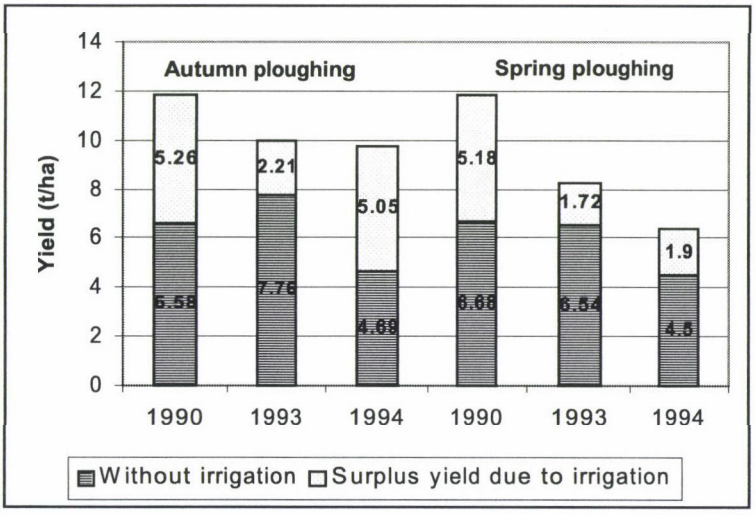

Fig. 1. Effect of soil cultivation and irrigation on maize yields in various years (t/ha). Debrecen 1990, 1993, 1994

before sowing and the almost fifty-day period without precipitation, as well as the long periods with daily maximum temperatures of over 30°C in July and August, greatly decreased the effectiveness of irrigation. After spring ploughing there was 1.9 t less yield per hectare than after autumn ploughing. We can only talk about modern, flexible soil cultivation, if the activities are adjusted to habitat, soil conditions, environmental factors and cultivation conditions.

In the present experiments, autumn ploughing with or without irrigation proved to be the most favourable soil treatment. The experimental results proved that autumn ploughing on chernozem soil, provided there was at least average precipitation, helped the formation of favourable soil conditions (unhindered growth of roots, good nutrient mobilization) and considerably increased the yield of maize. Averaged over the years examined, spring ploughing without irrigation did not differ significantly from the method where no ploughing was used. At the same time, in years of drought, the water preservation achieved without ploughing was significantly better than spring ploughing.

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PROPAGATION OF COMMON CARP (*CYPRINUS CARPIO*) AT A LARGE-SCALE HATCHERY IN HUNGARY

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Long-term data on the induced spawning of common carp (*Cyprinus carpio*) at a large-scale hatchery were evaluated. Breeders at the farm prefer medium-sized fish with body weights between 4 and 8 kg. The number of females selected for propagation in the first period of the spawning season made up about half of the total number of females (47.5%). The average spawning ratio from the data of 2620 females receiving hormonal injections was 79.8%. From the data of egg production of 2086 females, the average pseudogonadosomatic index (PGSI) has been calculated as 16.3 ± 5.87% (mean ± SD) for the same period. The time of induction has a significant effect on both female responsiveness and the relative quantity of eggs released. The spawning ratio was significantly lower in the third period of the reproductive season, compared to those in the first and second periods. The mean PGSI for the third period was significantly lower than that for the first. The considerable decrease in both reproductive parameters by the end of the spawning season is attributed to ovarian atresia. The spawning ratio and the mean PGSI for the mirror and scaled varieties were similar.

Key words: common carp, induced breeding, spawning ratio, pseudogonadosomatic index

Introduction

The common carp (*Cyprinus carpio*) is presently cultured as a domesticated fish species in most parts of Europe, all over Asia, and on a small scale in some countries of Africa and Latin America (Balon, 1995). In the intensive farming of common carp, effective seed production demands special techniques of artificial propagation. Induced spawning enables farmed broodstocks to ovulate under conditions of intensive culture, and allows the production of eggs to be adjusted as required to suit procedures on the farm. The technique, which uses pituitary extracts to induce ovulation, is known as hypophysation. Besides the advantage of regulating the time of spawning, hypophysation enables other methods of artificial propagation to be adopted, including hand-stripping, fertilization, incubation, hatching and larval rearing.

Although the hatchery production of common carp has been practised for more than three decades, studies on the results of induced breeding based on long-term data are non-existent. This is because routine hatchery work and precise data collection are hard to conduct simultaneously. Also, long-time storage of data collected under field conditions would be difficult to carry out.

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The Dinnyés Fish Hatchery and Farm, the production unit of the National Federation of Hungarian Fish Producers, was established in 1958. The farm was the first in the world to be equipped with an egg incubating and fry rearing facility for carp breeding using the Woynárovich method (Woynárovich, 1962) which has received worldwide acceptance (Huet, 1986). At present, the facility is capable of the simultaneous production and rearing of 150 million fish fry. The farm produces fry originating from genetically tested common carp stock, both mirror and scaled varieties. For the propagation of common carp, the standard technology of induced spawning has been applied (Woynárovich and Horváth, 1980).

In the present study, the following data on the induced spawning of common carp, collected between 1980 and 1997 at the Dinnyés Fish Hatchery and Farm, were analysed: i) composition of the broodstock on the basis of fish weight; ii) composition of the broodstock on the basis of time of induction; iii) response of females to hormonal treatment and relative quantity of stripped eggs expressed as the spawning ratio and the pseudogonadosomatic index (PGSI), respectively (their calculation will be shown later); spawning ratio and PGSI iv) relative to time of induction and v) relative to different varieties.

Materials and methods

Between 1980 and 1997, data on the following parameters were collected at the Dinnyés Fish Hatchery and Farm: number of common carp females selected for propagation, weight of the females, time of induction, response of females to hormonal treatment and the relative quantity of stripped eggs. The two latter reproductive parameters were expressed by the spawning ratio (# responsive females / # injected females) and PGSI, respectively. PGSI was calculated as follows: (weight of stripped egg mass / body weight before stripping) × 100 (Freund et al., 1995). The spawning ratio was analysed by the chi-square test ($P < 0.05$). PGSI data were subjected to one-way ANOVA ($P < 0.05$).

In examinations where the fish weight was concerned, except for the determination of PGSI (which is an individual reproductive parameter) weight categories were set up as follows: (3 kg, 4 kg, ..., 13 kg). Irrespective of the year of propagation, the females selected for breeding were classified into the appropriate category on the basis of their weight.

The time of induction was designated as follows: in each year, the reproductive season, lasting approximately four to six weeks, was divided into three periods of the same length of time. The time of induction of each female propagated in the season, independently of the year of propagation, was considered as an event in the first, the second or the third period.

In common carp, secondary sexual characteristics such as softening and rounding of the abdomen and the reddening and protrusion of the anal papilla and vent can be of help to breeders in selecting females ready for propagation (Horváth and Tamás, 1984). In large-scale breeding work at the Dinnyés Fish Hatchery and Farm, females ready for spawning are identified by employing this method. Less mature fish are returned to the holding pond for re-examination at a later date. Females showing the same signs of maturity are expected to exhibit similar responsiveness, irrespective of their body weights.

Results and discussion

1. Composition of the broodstock on the basis of fish weight

Between 1980 and 1997, 2620 common carp females were selected for propagation at the Dinnyés Fish Hatchery and Farm. The composition of the broodstock on the basis of fish weight is shown in Fig. 1. The number of females in three successive weight categories (5, 6 and 7 kg) comes close to half of the total number of females (48.3%). The proportion of females in the 4 kg and 8 kg weight categories each also exceeds 10%. The number of females with a body weight above 10 kg is relatively low. On the basis of these data, it can be concluded that breeders at the farm prefer medium-sized fish with body weights between 4 and 8 kg. These females are sexually mature and produce a relatively large quantity of eggs. They are also suitable for breeding purposes, since they are easier to handle during breeding procedures in the hatchery.

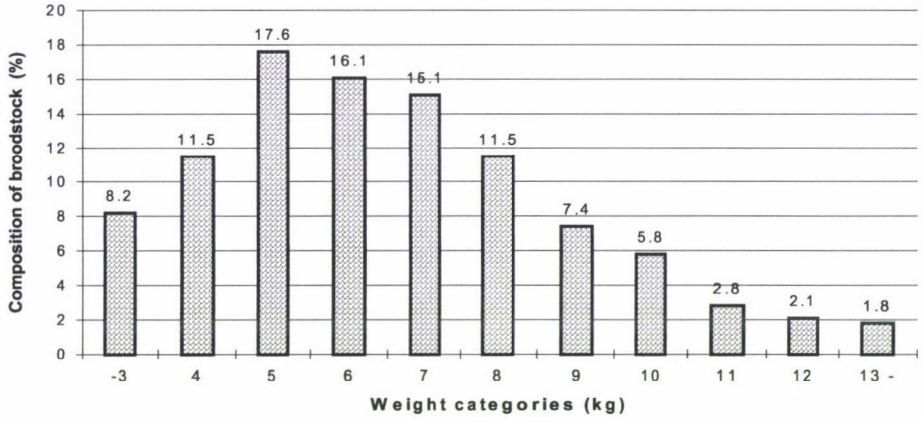

Fig. 1. Composition of the females selected for propagation between 1980 and 1997 at the Dinnyés Fish Hatchery and Farm on the basis of fish weight

2. Composition of the broodstock on the basis of the time of induction

The distribution of the common carp females selected for propagation between 1980 and 1997 over the first, second and third periods of the spawning season is shown in Fig. 2. The number of females selected for propagation in the first period of the spawning season is approximately half the total number of females (47.5%). The farm follows the strategy of producing as many swim-up larvae as possible early in the season, for safety reasons. The farm has the capacity to meet demands for swim-up larvae for their own use and for regular customers. Therefore, efforts are made to produce larvae to stock all the nursery ponds at the farm and as many larvae as are sure to be sold as early in the season as possible. The number of females selected for induced spawning in the second and third period of the season is relatively low, since the farm only performs occasional orders and re-stocks some nursery ponds from which advanced fry have been harvested earlier.

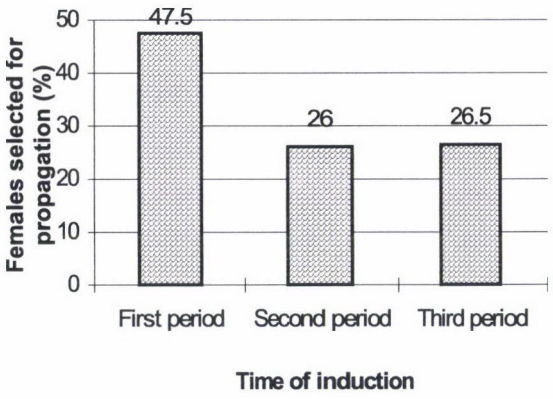

Fig. 2. Composition of the females selected for propagation between 1980 and 1997 at the Dinnyés Fish Hatchery and Farm on the basis of the time of induction

3. Response of females to hormonal treatment and relative quantity of stripped eggs

Between 1980 and 1997, 2091 of the 2620 carp females selected for propagation and treated with pituitary homogenate responded. The average spawning ratio over 18 spawning seasons was 79.8%. From the egg production data of 2,086 females, the average PGSI has been calculated as 16.3 ± 5.87% (mean ± SD) for the same period. The validity of these two indexes is supported by the fact that i) the standard technology of induced spawning of common carp was applied and ii) the indexes were calculated from a large number of data.

4. Response of females to hormonal treatment and relative quantity of stripped eggs as a function of the time of induction

The time of induction has a significant effect on both female responsiveness and the relative quantity of eggs released. The spawning ratio is significantly lower in the third period of the reproductive season, compared to those in the first and second periods (chi-square test; $\chi^2 = 25.3$) (Fig. 3). The mean PGSI for the third period was significantly lower than that of the first period (ANOVA; $F = 4.5$; $p = 0.011$) (Fig. 4). The considerable decrease in both reproductive parameters by the end of the spawning season is attributed to ovarian atresia. In holding ponds, due to the lack of environmental cues inducing spawning, final oocyte maturation and ovulation do not occur in the females. Instead, in the course of time, irreversible changes take place in the ovary. With the degeneration and resorption of oocytes, the relative quantity of stripped eggs decreases and finally the fish may lose its responsiveness to hormonal treatment.

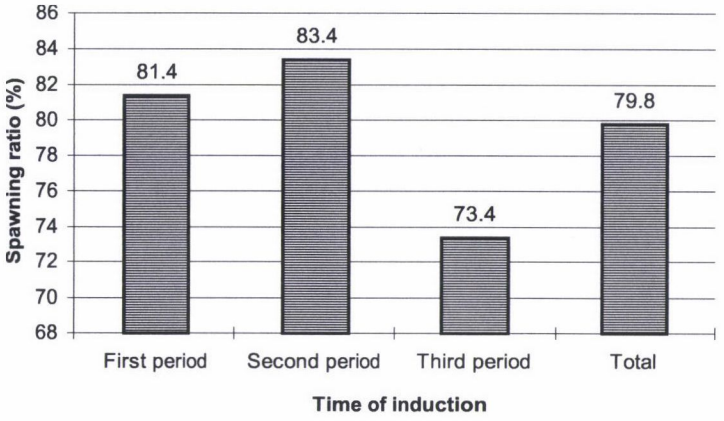

Fig. 3. Spawning ratio in the first, second and third periods of the spawning season and in the whole season disregarding the time of induction. The chi-square test indicated that the time of induction had a significant effect on the spawning ratio ($\chi^2 = 25.3$)

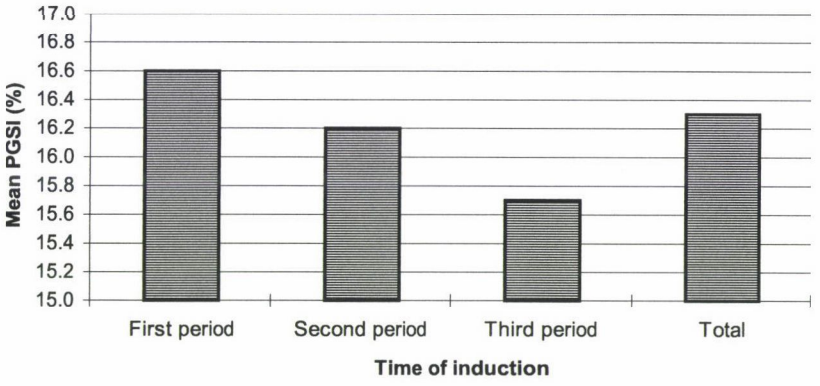

Fig. 4. Mean PGSI in the first, second and third periods of the spawning season and in the whole season disregarding the time of induction. ANOVA indicated that the time of induction had a significant effect on the relative quantity of stripped eggs ($F = 4.5$; $P = 0.011$). Groups designated by the same letter do not differ significantly from each other

5. Response of females to hormonal treatment and relative quantity of stripped eggs as a function of different varieties

Among the common carp females selected for propagation between 1980 and 1997 at the Dinnyés Fish Hatchery and Farm, 2,208 individuals, representing 84.9% of the whole population, belonged to the mirror variety. The 394 females of the scaled variety represented 15.1% of the common carp stock selected for induced spawning during the 18 breeding seasons. The percentages indicate the demand for carp fry and, above all, consumer habits.

The spawning ratio and the mean PGSI for the two different varieties were similar (Figs. 5 and 6). This fact indicates that the external characteristic of scaliness has no significant effect on such reproductive parameters as female responsiveness to hormonal treatment and relative egg production.

The above results will provide guidelines for carp breeders on the selection of optimal brood fish for spawning induction. It will also allow them to calculate their hatchery's larval production in advance. These results are helpful in calculating the total weight of broodstock which must be kept in order to produce a specific number of eggs.

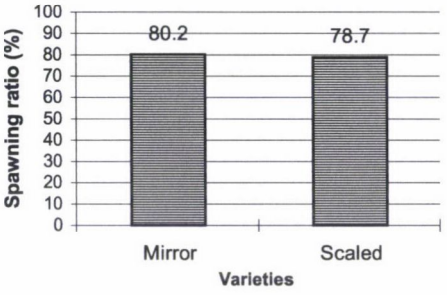

Fig. 5. Spawning ratios for the mirror and scaled varieties of common carp selected for propagation between 1980 and 1997 at the Dinnyés Fish Hatchery and Farm

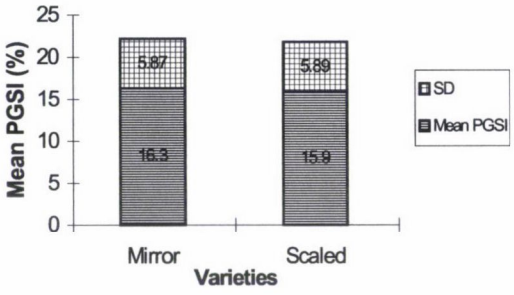

Fig. 6. PGSI (mean ± SD) for the mirror and scaled varieties of common carp selected for propagation between 1980 and 1997 at the Dinnyés Fish Hatchery and Farm

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Short communication

CALLUS INDUCTION AND PLANT REGENERATION IN DURUM WHEAT (*TRITICUM DURUM* L.)

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The objective of this research was to study the potential of three durum wheat cultivars for callus induction and plant regeneration using mature seeds. Seeds of the cultivars Hourani-27, Petra and ACSAD-65 were explanted onto agar solidified Murashige and Skoog (MS) medium containing different levels of 2,4-dichlorophenoxyacetic acid (2,4-D). Callus cultures were established from seeds of the three cultivars and gave the best results on MS medium supplemented with 2 and 3 mg l^{-1} 2,4-D. A maximum frequency of shoot formation (74%) was obtained by growing callus on medium supplemented with 2.0 mg l^{-1} 2,4-D. The highest rooting frequency was achieved by culturing callus on medium supplemented with 3.0 mg l^{-1} 2,4-D. The cultivar Petra showed significantly higher callus production and plant regeneration in comparison with the other two cultivars. The resultant plantlets were transferred either to fresh medium or to soil for further development.

Key words: *Triticum durum*, callus induction, plant regeneration

Introduction

Plant biotechnology techniques have emerged as an important aid to conventional breeding methods for rapid genetic improvement and for the integration of new genes into existing crop varieties. The success achieved in the regeneration of plants *in vitro* for a large number of cereal crops over the last decade (Morrish et al., 1987; Bhaskaran and Smith, 1990; Kyozuka et al., 1988; Redway et al., 1990; Vasil et al., 1990) has given an impetus for further work to improve the yield and production of crop species, especially wheat, which is considered a strategic crop in the developing countries.

To exploit *in vitro* selection and somaclonal variation, plant regeneration from cultured cells is a fundamental requirement for most applications of plant biotechnology (Brisibe et al., 1997). A number of factors, namely cultivar, explant, medium and cultural conditions, are known to affect the tissue culturing ability of crops (Raina, 1989). Even though callus induction and plant regeneration have been reported in a large number of graminous species (Morrish et al., 1987; Vasil, 1987; Denchev and Conger, 1994; Vasil et al., 1990; Redway et al., 1990), reports describing callus induction and plant regeneration in durum wheat are minimal. Durum wheat (*Triticum durum* L.) is one of the most important cereal crops in the Mediterranean regions of Europe, the Middle East and North Africa. It occupies about 70% of the total wheat area in this region (Nachit and Ketata, 1986).

The objective of this research was to study the potential of three durum wheat cultivars for callus induction and plant regeneration using mature seeds.

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Materials and methods

Seeds of three durum wheat cultivars, Hourani-27, Petra and ACSAD-65, which are commonly grown by farmers in Jordan, were used in this study. Seeds were obtained from the National Center for Agricultural Research and Technology Transfer (NCARTT), Amman, Jordan. Mature seeds of the three cultivars were surface sterilized for 15 min with 2.5% sodium hypochlorite, and then rinsed three times in sterile distilled water. The seeds were placed in plastic-capped glass jars (3 seeds/jar) containing 70 ml of MS medium (Murashige and Skoog, 1962) and incubated at 24±2°C in the dark to induce callus formation. The medium was solidified with 8 g l^{-1} agar and supplemented with 1.0, 2.0, 3.0 or 4.0 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) and 30 g l^{-1} sucrose. The pH of the medium was adjusted to 5.8 before agar application, and all the medium components were sterilized by autoclaving at 126°C and 15 bars for 20 min. After 5 weeks, the calli were separated from the endosperm and the callus initiated (250±25 mg) was subcultured into new jars with fresh proliferation medium containing the following treatments: 1.0, 2.0, 3.0 or 4.0 mg l^{-1} 2,4-D. Contaminated cultures and brown coloured cultures were discarded. The cultures were incubated in a culture room at 24±2°C with 16 h light (photosynthetically active radiation = 40–60 µmol m^{-2} s^{-1}) provided by cool white fluorescent bulbs. After 5 weeks, the regeneration potential (shoot and root formation) was studied. Depending on their size, the resultant plantlets were transferred either to fresh MS medium or to soil for further development.

The experimental design was completely randomized with a factorial arrangement of treatments, with the 2,4-D levels as main plots and the cultivars as subplots with seven replications. The data were statistically analysed according to Steel and Torrie (1980) for LSD ($P<0.05$).

Results and discussion

Calli were established from the embryos of mature wheat seeds. In 30 to 40 days, one jar containing three seeds was able to produce a minimum of 1 g of calli in all the cultivars regardless of the 2,4-D level in the culture medium. The callus induction frequencies are given in Tables 1 and 2. Large, significant differences were noted for callus formation between different levels of 2,4-D in the culture medium. Of the four levels used, medium supplemented with 2.0 mg l^{-1} 2,4-D induced the highest frequency of callus formation, averaged over the cultivars. A low frequency of callus formation was recorded on medium supplemented with 1.0 or 4.0 mg l^{-1} 2,4-D, regardless of the cultivar. 2,4-D has been found effective for callus induction in many cereal crops (Vishnoi and Kothari, 1996; Eapen and George, 1989; Bhaskaran and Smith, 1990).

The cultivars showed significant differences with respect to callus formation regardless of the level of 2,4-D applied in the culture medium. The cultivar Petra attained a significantly greater frequency of callus formation than the other two cultivars at all levels of 2,4-D in the culture medium (Table 2). ACSAD-65 attained a higher frequency of callus formation than Hourani-27 on medium supplemented with 2.0 mg l^{-1} 2,4-D, while the opposite occurred on medium supplemented with 3.0 mg l^{-1} 2,4-D, when Hourani-27 attained a higher frequency of callus formation than ACSAD-65. Both cultivars (Hourani-27 and ACSAD-65) showed similar frequencies of callus formation on medium supplemented with 1.0 and 4.0 mg l^{-1} 2,4-D (Table 2).

Table 1
Main effects of 2,4-dichlorophenoxyacetic acid (2,4-D) and cultivar on callus induction, shoot and root formation of durum wheat grown *in vitro*

	Response		
	Callus induction (%)	Shoot formation (%)	Root formation (%)
2,4-D (mg/l)			
1.0	26.0c*	32.0c	30.7c
2.0	59.3a	73.7a	33.7c
3.0	60.0a	51.7b	67.9a
4.0	46.7b	36.3c	58.3b
Cultivar			
Hourani-27	39.4b	37.0b	48.8b
Petra	65.2a	54.8a	55.7a
ACSAD-65	39.2b	52.8a	38.5c

*Values within a column followed by the same letter are not different (P>0.05) according to the LSD test

Table 2
Interactive effects of 2,4-dichlorophenoxyacetic acid (2,4-D) and cultivar on callus induction, shoot and root formation of durum wheat grown *in vitro*

2,4-D (mg/l)	Cultivar	Response		
		Callus induction (%)	Shoot formation (%)	Root formation (%)
1.0	Hourani-27	19.0d*	19.0d	36.0c
	Petra	41.0c	39.0c	38.0c
	ACSAD-65	18.0d	38.0c	18.0d
2.0	Hourani-27	38.0c	56.0b	41.0c
	Petra	81.0a	83.0a	40.0c
	ACSAD-65	59.0b	79.0a	20.0d
3.0	Hourani-27	60.7b	38.0c	60.0b
	Petra	78.0a	59.0b	82.7a
	ACSAD-65	41.0c	58.0b	61.0b
4.0	Hourani-27	40.0c	35.0c	58.0b
	Petra	61.0b	38.0c	62.0b
	ACSAD-65	39.0c	36.0c	55.0b

*Values within a column followed by the same letter are not different (P>0.05) according to the LSD test

Upon transfer to plantlet regeneration medium, the subcultured calli became compact and greenish due to the differentiation of the plantlets. It can be seen from the results presented in Tables 1 and 2 that all four levels of 2,4-D used to supplement the medium promoted the development of shoots and roots. Calli regenerated on culture medium supplemented with 2.0 mg l^{-1} 2,4-D exhibited the highest frequency of shoot formation, while calli regenerated on

culture medium supplemented with 3.0 mg l^{-1} 2,4-D exhibited the highest frequency of root formation. 2,4-D has been found effective for inducing organogenesis in many cereal crops (Vishnoi and Kothari, 1996; Eapen and George, 1989; Denchev and Conger, 1994).

The frequency of plant regeneration was greatly influenced by the cultivar. Averaged over 2,4-D levels, Petra attained a greater frequency of shoot and root formation than the other two cultivars, even though the difference between Petra and ACSAD-65 was not significant for root formation frequency (Table 2). Hourani-27 attained a lower frequency of shoot formation than the other two cultivars on medium supplemented with 1.0, 2.0 or 3.0 mg l^{-1} 2,4-D (Table 2). No significant differences were noted between Petra and ACSAD-65 for shoot formation frequency on medium supplemented with various levels of 2,4-D. Petra attained a significantly greater frequency of root formation than other cultivars on medium supplemented with 3.0 mg l^{-1} 2,4-D (Table 2). ACSAD-65 attained a lower frequency of root formation than the other two cultivars on medium supplemented with 1.0 and 2.0 mg l^{-1} 2,4-D. Genotypic differences in plant regeneration potential have also been reported for other cereal crops (Brisibe et al., 1997; Kyozuka et al., 1988; Yang et al., 1991).

The results of this study indicated that callus induction and plant regeneration potential are highly dependent upon the cultivar and the level of 2,4-D in the culture medium. It was possible to regenerate a large number of plants from mature seeds of durum wheat. These plants will be transferred to the field and will be evaluated for vigour, biomass production and other traits.

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Review

TIMING IT RIGHT: THE MEASUREMENT AND PREDICTION OF FLOWERING

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Although the model described here was developed from research in controlled environments, there is now considerable evidence that it can be applied to a very wide range of natural environments in several species. Multi-locational trials augmented by successional sowings and, if considered necessary, supplementary illumination in the field to increase daylength, can be used to estimate the values of the model coefficients: (1) to characterize germplasm collections and so predict flowering behaviour elsewhere; (2) for interpreting and understanding crop adaptation; and (3) for genetic analysis of photoperiod sensitivity. We do not yet know whether the model has any contribution to make to the understanding of the biochemical mechanisms of photoperiod and temperature responses, but at the very least, it should provide the basis for indicating the most appropriate environmental conditions, genotypes and physiological stage of the plants most suitable for such investigations.

Key words: prediction of flowering, modelling, annual crops

Introduction

Annual cycles of behaviour of plants and animals are strongly influenced by the environment. For example, responses to environmental cues control the timing of reproductive cycles, bud dormancy, hibernation, migration, diapause and other seasonal activities.

The limits to the growing season in temperate latitudes are determined mainly by temperature: in the winter the temperature is too cold. In the tropics growth is seldom limited by cold temperatures (except at high altitude), but it is commonly limited by lack of water since there is often a distinct dry season. In both cases the appropriate survival strategy for many plants, and especially those grown as annual crops, is to accumulate as much photosynthate as possible by exploiting most of the growing season, and then to flower, fruit and mature before adverse conditions set in.

Plants and animals seldom respond directly to these environmental changes – probably because they are not reliable. To start growing in mid-winter in response to a temporary warm spell, for example, could be disastrous. It is better to respond to a signal which more reliably times development in relation to the seasons. The most common one used in order to control seasonal behaviour is daylength, because the daylength on any date at any latitude is

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precisely predictable. In addition to daylength, plants also utilise accumulated temperature (e.g. day degrees above a particular base value). This ability to integrate temperature with time enables them to respond to a 'climatic' feature rather than to a 'weather' signal as would be the case if they responded immediately to a particular threshold temperature. In many cases, the primary photoperiodic response can, if necessary, be fine-tuned so as to adjust behaviour to significant annual variations in seasonal temperature.

There is at least some information on the photothermal flowering responses of thousands of species, albeit with a strong bias towards photoperiodism. Physiological signals that induce or inhibit flowering have been described; metabolic mutants in which the response to inductive daylengths is altered are increasingly targeted; but progress in understanding the physiological, genetic and molecular bases of induction has been slow. Intense research efforts have often focussed on a single clone or cultivar; experiments are commonly done at a single temperature; and there is still no means of assaying the quantity of stimulus (to flower or not to flower) produced in response to a single treatment.

Given these constraints, and the range and variety of flowering responses to photothermal conditions it has proved difficult to find patterns or to make generalizations that have some meaning and predictive value. I do not wish to lose sight of that range of responses and their ecological significance. Nevertheless, I shall attempt a synthesis and suggest generalizations. Indeed, practical utility demands both simplicity and a proven ability to anticipate biological events.

The timing of flowering and climatic adaptation

People probably began to domesticate plants about 10,000 years ago and more or less simultaneously in at least three different regions – the Near East, northern China and Meso-America. Thus, agriculture began, and other crops were later domesticated in regions with pronounced wet and dry seasons, neither of which were particularly long. By the time 'seed agriculture' had come to replace 'colonization and gathering' it must have been clear that crops could only be grown satisfactorily during relatively short, wetter seasons.

In discussing the genetic adaptation of field crops to the variable and frequently stressful conditions under which they are grown, the key aim is to optimise productivity by matching the ontogeny [sequence of developmental stages] to the weather resources of the environment [e.g. duration of favourable temperature or water supply] and, where unfavourable extremes are unavoidable, to minimise their coincidence with more vulnerable stages. Not surprisingly, therefore, phenology [the influence of environment on ontogeny] is the most important single factor influencing genotypic adaptation.

Flowering is a particularly important event in crop development since it is a phase which is especially vulnerable to environmental stress. Furthermore it is the timing of this stage of plant development that is very largely responsible for

determining when cereal, pulse, oilseed crops and many vegetables will subsequently be ripe for harvest. It was 'fairly certain' about fifty years ago that a given genotype of any annual crop has its own definite optimal requirements of temperature and light [photoperiod] without which it cannot proceed at an economically desirable rate to flower formation, flowering and the production of seeds. Later, the photothermal control of the timing of flowering came to be recognized as 'especially important in crop adaptation'. Since then, however, successive attempts to correlate the timing of flowering in a wide range of crops with various weather variables have tended to confirm the viewpoint that the problem of flowering is a very complicated phenomenon, and there is little to be gained by considering it simple. Indeed, because the reliable prediction of flowering in fluctuating field environments has proved so difficult, a common viewpoint has been that photothermal effects are strongly curvilinear and interactive. However, I now believe that the responses are in fact amenable to relatively simple analysis to provide predictive models which are valuable in breeding, agronomy and genetics.

The ecogeography of photothermal responses

Because the earth's axis is inclined at 23.5° towards the plane of the earth's orbit around the sun, there is a regular annual variation in daylength in any part of the world, although the extent of that variation depends on latitude (Table 1). Given the relatively small seasonal differences in tropical daylengths it is not surprising that some of the most sensitive flowering responses to photoperiod are found in tropical species: differences of 10–20 min day^{-1} can be critical in rice (*Oryza sativa*) and cowpea (*Vigna unguiculata*).

Table 1
Variation in daylength (h:min) with latitude

Latitude (°N or °S) with exemplar locations		Daylength inclusive of Civil Twilight (h:min)*		
		Longest	Shortest	Annual variation
0	Entebbe, Uganda	12:50	12:50	0:00
10	Costa Rica	13:29	12:19	1:10
20	Hawaii	14:11	11:44	2:27
30	Cairo	15:01	11:04	3:57
40	Philadelphia, USA	16:01	10:22	5:39
50	South Cornwall, UK	17:53	9:20	8:33
60	Shetland Isles	22:25	7:48	14:37
70	North Alaska	24:00	0:00	24:00

*Civil Twilight begins before sunrise and ends after sunset when the true centre of the sun is 6° below the horizon; it corresponds to an illuminance of about 4 lux

Current knowledge indicates that all crops of tropical or subtropical origin are essentially short-day plants (SDPs), whereas the vast majority of crops of Mediterranean or temperate origin are long-day plants (LDPs). The only exceptions to this generalization of which I am aware are the SDPs sunflower (*Helianthus annuus*) and soyabean (*Glycine max*) which originated between 30° and 35°N in the USA and between 34° and 40°N in China, respectively. The exception of soyabean can be explained by a major perturbation of the intertropical convergence zone (ITCZ) in summer in north-east China which results in a late growing season.

Many species of crops also include genotypes which are insensitive to photoperiod, i.e. day neutral plants (DNPs), but these are often the product of special selection – either deliberate, or as an inevitable consequence of adaptation to regions outside the latitude of origin. For example, given that tropical plants typically have short-day responses flowering would be excessively delayed in the long days of temperate summers unless, in the course of their spread from the low latitudes, strains were selected which were much less sensitive or insensitive to photoperiod. Conversely, species of temperate origin with long-day responses could not adapt to the short days of the tropics unless strains with little or no sensitivity were selected. Thus the rice landraces of tropical origin (*Oryza sativa* spp. *indica*) are typically very sensitive SDPs while those adapted to the temperate latitudes of Japan, Europe and USA (spp. *japonica*) are typically much less sensitive or insensitive. Examples from vegetables are not always so clear but tomato (*Lycopersicon esculentum*), for example, was probably domesticated in Central America and is essentially a short-day species but those cultivars in commercial production tend to be relatively insensitive to daylength, probably as a result of intensive breeding in temperate latitudes. The same applies to common bean (*Phaseolus vulgaris)*. On the other hand the photoperiod sensitivity of lentil (*Lens culinaris*) which is a long-day species originating in West Asia is less in tropical landraces. The same applies for wheat (*Triticum aestivum*).

Another feature in the control of flowering is that many LDP species include genotypes in which flowering is advanced by cool temperatures – a response described as vernalization. Such a response appears to be extremely rare amongst SDPs , and in fact, only one confirmed example is known, namely *Chrysanthemum moriflorum*, which originated in China between 20 and 35°N.

It is plausible that SDPs are typical of the tropics because the growing season is limited by rain (not by temperature or irradiance) and that the rainy season is a product of the ITCZ. This zone tends to coincide with the latitude where the sun is overhead at midday, and the sun's zenith moves from 23.5°N in June to 23.5°S in December. Crops ideally ripen towards the end of the rainy season after the accumulation of sufficient vegetative growth, and when the conditions are becoming drier and more suitable for the maturation of seeds. In these circumstances only a short-day response could result in appropriate phenology.

In Mediterranean and temperate latitudes it is also an advantage for crops to ripen towards the end of the growing season, and in a warm and not unduly wet season for seed maturation. The growing season in these regions is primarily limited by cool temperatures and, to a lesser extent, by low irradiance during the winter. In Mediterranean climates it is also curtailed by summer drought. Accordingly most crops flower in the spring in the Mediterranean and in the early summer in temperate latitudes – in both cases when the days are lengthening. Under these circumstances, only a long-day response can ensure appropriate phenology; and in the case of autumn-sown annuals or biennials, this long-day response needs to be supplemented by a prior vernalization requirement in order to prevent the plants responding to the relatively long days of autumn.

In spite of the fact that plant photoperiodism was discovered more than 80 years ago and has been widely researched since then, the mechanisms at the molecular and biochemical level are still largely a mystery. Indeed, flowering in LDPs is not a simple mirror image of the SDP system; unlike SDPs, where night length is of overriding importance, light quality and quantity during the photoperiod are of considerable importance in LDPs. In both responses we know that the phytochrome pigment is involved. There is evidence too for substances which can be transmitted from leaves which either promote or inhibit flowering, but nothing is known of their structure or properties. In terms of fundamental understanding, animal photoperiodism fares no better.

A quantitative model to predict flowering

Luckily, it is not necessary to understand the underlying mechanisms in order to quantify and predict the flowering responses of crops. Indeed, there is no guarantee that a knowledge of the molecular events would help to predict and quantify the overall response. [In passing, just think of the enormous amount of detail which is known about the molecular and biochemical mechanisms of photosynthesis, so fundamental to plant growth, yet none of this knowledge has so far helped in crop improvement.]

In developing a quantitative flowering prediction model my research colleagues and I (see Acknowledgements) began from the concept that most responses to temperature in chemistry or physiology are best considered as rates. In the case of flowering, we are concerned with the rate at which the plant develops through the vegetative phase until a meristem changes its anatomy and function to become reproductive (flower initiation) or until mature flowers are visible (flowering). That rate cannot be measured directly but, like an enzyme reaction rate, it can be inferred by taking the reciprocal of the time taken for the end-point to be reached. So, although we record the time in days taken from sowing to flowering (f), and this is what we are ultimately concerned to predict, I concentrate here on the more fundamental property of rate of progress towards this event ($1/f$).

The main advantages of this approach are as follows:

1. Typically the separate responses to both photoperiod and temperature become linear over wide ranges of conditions if phenology data are transformed to rates.

2. Whereas large, significant interactions occur between temperature and photoperiod responses if data are analysed as time to flowering, these interactions often disappear if rates are used.

3. A consequence of (1) and (2) is that simple equations without interaction terms within certain well-defined limits can be developed to predict rates of progress towards flowering and therefore, indirectly, the times taken from sowing to flowering.

4. As there are no interaction terms in such equations if it possible to identify and measure the separate genetic control of photoperiod-sensitivity and of temperature-sensitivity.

5. The recognition that linearized rate equations may be applied to the data permits the application of the concept of thermal time (for photoperiod-insensitive genotypes) or an analogous concept of photothermal time (for photoperiod-sensitive genotypes). The use of these concepts allows the prediction of times to flowering in environments which are not constant, e.g. in natural environments where temperature fluctuates and photoperiod changes systematically. It is not always realised that the concepts of thermal time and photothermal time are *only* legitimate if *rates* of development are linearly related to temperature and/or photoperiod.

6. As a consequence of (3) and (4), the genotypically controlled values of the parameters which describe sensitivity to photoperiod and temperature can, where appropriate models have been developed, be estimated from responses in only a few controlled, but carefully chosen, environments (in some cases, in theory, no more than four may be essential). As a consequence of (5), it is possible to use fluctuating field conditions, or to integrate the use of controlled and natural environments to estimate these parameters.

7. A consequence of (4) and (6) is that simple and economic techniques may prove feasible for screening large germplasm collections for sensitivity to photoperiod and temperature in a way which allows rational genetic analysis and predictions of time to flowering in a wide range of environments.

The experimental results and arguments which led to these conclusions are described in detail elsewhere (see References, Fig. 1). Only the principal features of the photothermal models are summarized here (Box 1) and illustrated for soyabean in Figures 1a, b.

The three intersecting planes described by equations 1, 2 and 3, which meet at the boundaries defined by equations 4, 5 and 6, form the triple-plane rate model of development which has now been shown to be applicable to a very wide range of species, e.g. cowpea; soyabean; mung bean (*Vigna radiata*) and related *Vigna* spp.; Bambara groundnut (*Vigna subterranea*); common bean; faba bean (*Vicia faba*); pea (*Pisum sativum*); chickpea (*Cicer arietinum*); subterranean clover (*Trifolium subterraneum*); barley; wheat; rice and lentil.

Box 1. The general triple-plane rate model of flowering response to the photothermal environment (as illustrated in Figure 1a, b).

There are three basic equations (1, 2 and 3 below) which together define the time taken to flower in any environment (except in supra-optimal temperatures or when other stresses are severe). The equations involve six genotypic coefficients (a, b, a', b', c', d') all of which have identifiable biological significance and have values which are independent of the environment.

In photoperiod-insensitive plants (Fig. 1a), or in daylengths shorter than the critical daylength P_c in short day plants (plane B in Fig. 1b) or in daylengths longer than P_c in long day plants:

$$1/f = a + bT \qquad (1)$$

where f is the time from sowing to first flower (days) and T is mean temperature (°C).

Between the critical and ceiling photoperiods (defined below) the relation in photoperiod-sensitive genotypes is:

$$1/f = a' + b'T + c'P \qquad (2)$$

where P is mean photoperiod (hd^{-1}) and where c' has a positive value in long day plants but is negative in short day plants (plane C in Fig. 1b).

The maximum delay in flowering is reached at the ceiling photoperiod, P_{ce} above which in short day plants, or below which in long day plants there is no further delay in flowering. In short day plants, at least, when $P \geq P_{ce}$ variations in either P or T do not affect f, and so (plane D in Fig. 1b):

$$1/f = d' \qquad (3)$$

The critical and ceiling photoperiods mark boundaries between the three planes (Fig. 1b) and are given by:

$$P_c = [a - a' + T(b - b')]/c' \qquad (4)$$

and by

$$P_{ce} = [d' - (a' + b'T)]/c' \qquad (5)$$

and two of the temperature-determined boundaries to the planes are given by:

$$T_p = (-a + d')/b \qquad (6)$$

where T_p is the temperature above which photoperiod-sensitivity genes are expressed, and by:

$$T_b = -a/b \qquad (7)$$

where T_b is the base temperature below which it is too cool for progress to flowering to occur

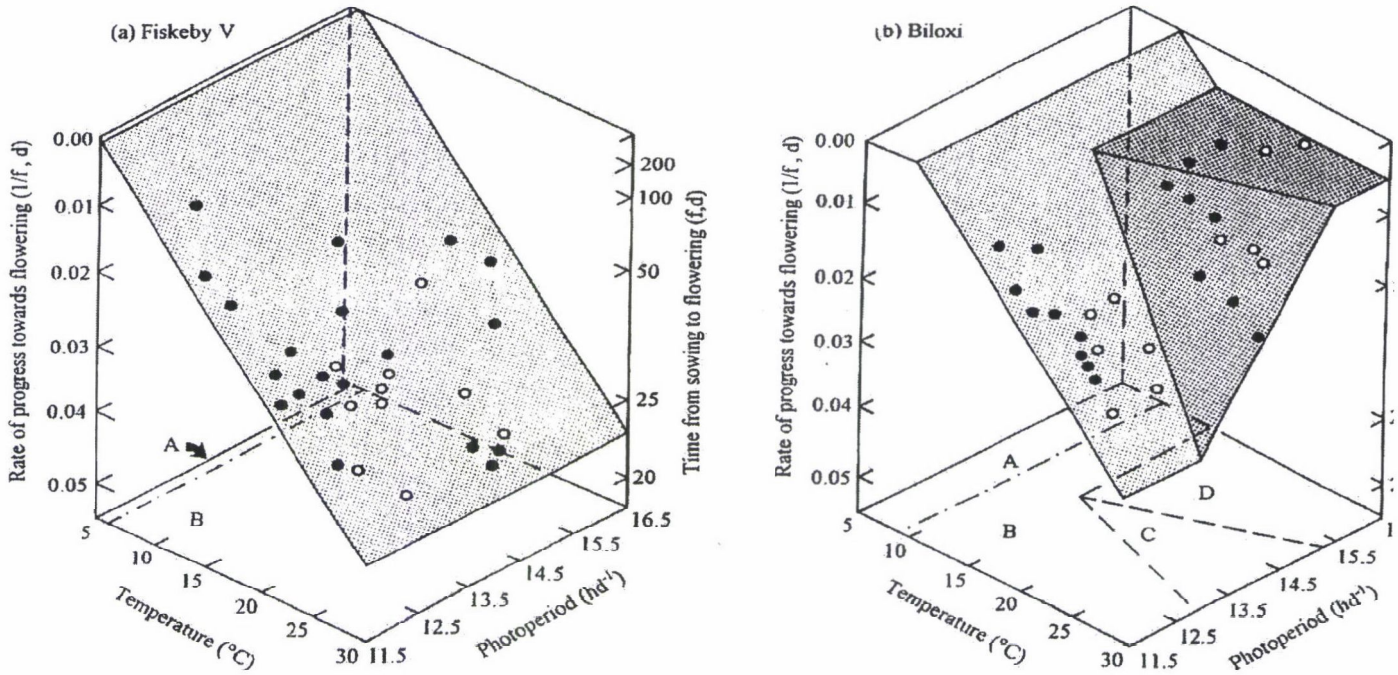

Fig. 1. Photothermal flowering responses for two genotypes (a) Fiskeby V and (b) Biloxi of the SDP soyabean, determined from observations from crops sown on various dates at six sites in Australia in 1986–1988 (●) and at one site in Australia and two in Taiwan in 1989–1990 (O). The vertical scale ($1/f$) on the left hand ordinate is converted to f as a non-linear scale on the right of each figure. The base of each graph has been divided into up to four sectors by vertically projecting: (A) the domain of environments where flowering cannot occur ($1/f$=0), because the temperature is too cool; (B) where the time taken to flower depends on temperature only; (C) the domain of environments where both photoperiod and temperature independently control the time taken to flower; and (D) the domain of environments in which photoperiodic delay is maximal and where variations in photoperiod or temperatures have no effect. The line between sectors B and C represents the critical photoperiod and that between C and D represents the ceiling photoperiod (From Summerfield et al. 1993)

In some cases only one of the three planes of the model is required, e.g. only the thermal plane (equation 1) is needed when dealing with photoperiod-insensitive plants at suboptimal temperatures. In other cases in photoperiod-sensitive plants it may be that the range of relevant natural environments falls mostly within the photothermal plane (equation 2), as has been found when applying the model to lentil.

In some cases, however, the range of conditions which have to be considered spreads over two or three of the planes, and when dealing with multi-location sowing date trials there is a potential difficulty of deciding which observations near a boundary should be allocated to each plane on either side of it. However, the problem has been solved statistically and operationally by the development of an iterative computer procedure called RoDMod. Examples of applying this to field data for soyabean are shown in Fig. 1b.

Figure 1b demonstrates that the photothermal environment can be divided into different domains according to their influence on development. In two of these planes (the thermal and photothermal) there is ample evidence that the response to temperature is linear; whereas in the other (the plane of maximum delay) there is no response in soyabean. There is also considerable evidence that the rate of progress of a number of developmental processes is a linear function of temperature. The response has been examined in greatest detail for seed germination. It is by no means clear why the response of developmental rate to temperature between the base and optimum values should be linear, even though there has been some speculation. It is even less clear why the rate of progress towards flowering should be a linear function of photoperiod between the critical and ceiling values. But it is a fortunate circumstance that reponse to both temperature and photoperiod within any domain are linear and without interaction since this simplified the mathematics. If the raw data, i.e. times from sowing to first flower, are not transformed to rate then clearly powerful interactions occur. The transformation to rates removes the need for an interaction term and also has another statistical advantage: times to flower show non-homogeneous variance – the variance increasing with delay in flowering – a common observation of experimentalists. Transformation to rates removes this problem. Not only does the use of rates legitimize the statistical analysis of responses but it also takes the investigation nearer to the underlying physiology, for it is difficult to avoid the deduction that the time taken to reach an endpoint such as flowering depends on the rate of the processes leading to it. The converse would be illogical.

Although these analytical and theoretical advantages of using a model based on rates are important, the main virtue of the triple-plane rate model is that it involves relatively few coefficients (a,b,a',b',c',d'), all of which, and their derivatives, have clearly defined biological meaning (e.g. c' indicates relative photoperiod sensitivity; a/b is the base temperature) and, of paramount importance, the six coefficients are not affected by the environment but are genotypic characters which determine phenotypic response to the environment, and which can predict this response quantitatively.

Although it is possible to investigate the genetics of photoperiodism without a knowledge of the quantitative nature of the photoperiod and temperature responses outlined here, the results could be – and in some cases have been – misleading.

For example, if photoperiod sensitivity were to be assessed based on the difference in time to flowering in two contrasting daylengths, the value obtained would depend not only on temperature but also on whether the two photoperiods selected are within environmental domain B or one observation is in domain A or C. Even more problems of interpretation can arise, as they often did in earlier work, if comparisons are made on the basis of two photoperiodic regimes in which the number of plants which have flowered after an arbitrary time are recorded, rather than measuring the time taken for each sample to flower, from which rates can be derived. Furthermore, since temperature as well as photoperiod affects the flowering responses, it is important to compare responses at different temperatures to determine whether or not a gene which influences photoperiod sensitivity also affects the response, or is affected by the response, to temperature.

In the soyabean genome five so-called maturity loci have been identified with two alternative alleles at each locus, viz. E_1/e_1, E_2/e_2, E_3/e_3, E_4/e_4 and E_5/e_5. We have investigated the effects of all eight possible isolines of three of these loci E_1/e_1, E_2/e_2 and E_3/e_3 in a cv. Clark background. (cv. Clark has the following complement of maturity genes: $e_1\ E_2\ E_3\ E_4\ e_5$.) It was shown that none of the genes affects temperature sensitivity (b or b') and that the major effect of the dominant alleles is on photoperiod sensitivity, i.e. on c', although additional (pleiotropic?) effects can also be recognised. There were three categories of increasing sensitivity to photoperiod: (1) least sensitivity (but nevertheless still slightly sensitive, probably due to the presence of E_4 in the cv. Clark background) represented by $e_1\ e_2\ e_3$, $e_1\ E_2\ e_3$ and $e_1\ e_2\ E_3$; (2) intermediate sensitivity represented by $E_1\ e_2\ e_3$ and $e_1\ E_2\ E_3$; and (3) most sensitivity represented by $E_1\ E_2\ e_3$, $E_1\ e_2\ E_3$ and $E_1\ E_2\ E_3$. Thus, photoperiod sensitivity is dominant. Increase in sensitivity never accelerates flowering, but delays it in any photoperiod greater than the critical photoperiod. E_1 has the greatest effect. While neither E_2 nor E_3 have any effect independently, together they show epistasis and produce roughly the same effect as E_1 on its own. Furthermore, either E_2 or E_3 can enhance the effect of E_1.

With the increase in photoperiod sensitivity induced by these gene combinations, there is also a concommitant decrease in the value of d', i.e. an increase in the time taken to flower in unfavourable photoperiods greater than the ceiling value. The maximum time taken to flower (at a mean temperature of 25°C) in these isolines varies from about 50 days in the least sensitive genotypes to about 100 days in the most sensitive one.

All these effects of the *E*-maturity genes which result in different phenotypic responses in different environments are genetically characterised mostly by the correlated values of the c' and d' coefficients, although a' is also affected. We believe, however, that the most important effects of the genes for practical applications reside in the values of the c' and d' coefficients and, since the values are correlated, it might well prove adequate when screening germplasm to concentrate on c', thus reducing the number of environments required.

Although the model described here was developed from research in controlled environments, there is now considerable evidence that it can be applied to a very wide range of natural environments in several species. Multi-locational trials augmented by successional sowings and, if considered necessary, supplementary illumination in the field to increase daylength, can be used to estimate the values of the model coefficients: (1) to characterize germplasm collections and so predict flowering behaviour elsewhere; (2) for interpreting and understanding crop adaptation; and (3) for genetic analysis of photoperiod sensitivity. We do not yet know whether the model has any contribution to make to the understanding of the biochemical mechanisms of photoperiod and temperature responses, but at the very least, it should provide the basis for indicating the most appropriate environmental conditions, genotypes and physiological stage of the plants most suitable for such investigations.

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Review

MAGNESIUM RESEARCH IN HUNGARIAN AGRICULTURE[+]

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In Hungary research on Mg has been underway for several decades in the fields of crop production and animal husbandry. This paper gives an overview of the results achieved concerning the effects of Mg fertilization and dolomite-supplemented N fertilizer application on yields. The presentation of agrochemistry research discusses the development of soil Mg tests and a description of the Mg supply in soil types found in Hungary, based on national soil analysis results. The determination of optimal K, Ca and Mg rates and the use of dolomite to ameliorate acidic soils with low Mg contents are also discussed.

The principal objectives of Mg research connected with farm animals were to improve the utilization of feeds and the productivity, fertility and health status of farm animals (cows, heifers, pigs and broilers), and to study the role of Mg in biological and biochemical processes.

Key words: magnesium in soils, magnesium fertilization, plant quantity and quality, magnesium supplementation and metabolism of animals

Agrochemistry and plant production

The first Mg fertilization field experiment in Hungary was conducted by Kemenesy and Nyéki (1963) on brown forest soil in Somogy county. Mg fertilization increased the yield and starch content of potatoes. Bocz (1962) achieved positive results in sugarbeet grown on chernozem. Láng (1973) examined the effect of NPK and Mg fertilization on rye and potatoes grown on brown forest soil with alternating thin layers of clay in long-term experiments. He found that in certain years over the average of ten years, potato yields were increased by Mg fertilization.

The findings of the above researchers provided the impetus for further experiments in plant production.

Parallel with the research in plant production, agrochemical experiments were conducted to develop methods for investigating soil and plant Mg contents. They also included pot experiments to examine factors affecting the efficiency of Mg fertilization, while a description of the Mg supplies of soils in Hungary was also provided (Loch 1970; 1975; 1985).

[+]On the basis of a paper presented at the 6th European Magnesium Congress, Budapest, May 13–16, 1998

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Kiss (1972, 1977) developed two dolomite-containing N fertilizers *AGRONIT* and *KARDONIT*. Applied to acidic soils, both fertilizers improved the yield quantity and quality, and increased the resistance of the crop to fungal plant diseases.

The positive NPK balance in the soils established by the 1970s urged the need for the examination of nutrient elements on a broader scale. Within the framework of the national nutrient analysis programme, the measurement of Mg in 1.0 M KCl soil extracts was introduced as a new method based on the comparative investigations of Loch (1970) (Table 1).

Table 1
Limiting values of the magnesium supply in soils (measured in 1 mol/dm^3 KCl extraction solution)

K_A*	Mechanical composition	Mg mg/kg		
		poor	fair	good
<30	sand	< 40	40 – 60	> 60
31–42	sandy loam, loam	< 60	60 – 00	> 100
<43	clayey loam, clay	< 100	100– 200	> 200

*K_A = amount of water (ml) which can be taken up by 100 g soil before the plasticity level is attained (Arany number)

Examinations carried out nationwide show that the readily available Mg content of soils is largely dependent upon their physical and chemical properties and upon the conditions under which they were formed. Soils deficient in Mg are usually acidic soils with a poor supply of organic and inorganic colloids and low adsorption capacity. They are mostly ranked as blown sand or sand with low humus content. Leached types of brown forest soil are also poor in Mg. Chernozem, meadow and swamp soils, on the other hand, are rich in Mg. The results of the investigations show that almost 90% of the soils nationwide are well supplied with Mg (Loch and Buzásné, 1992).

Loch et al. (1986) conducted pot experiments to examine the impact of K-Ca-Mg doses and ratios on plant Mg uptake. They found that one-sided treatments of acidic soils with a Mg shortage, such as liming or the application of high K doses, produced undesirable results. For the definition of optimum doses and ratios, the Box-Wilson method was adapted and further developed (Loch, 1990) (Fig. 1a, b, c, d)

An incorrect ratio of exchangeable cations in the soil will also act as an inhibitor of plant Mg uptake on soils with high Mg supplies. This finding is supported by results gained in experiments on sugarbeet and sunflower grown on chernozem, where the sugar and oil contents were increased by Mg top dressing (Loch et al., 1994).

Increasing the magnesium supply by chemical soil improvement

In acidic soils with a shortage of Mg, chemical amendments that contain significant amounts of magnesium have more favourable effects on the chemical properties of the soil than liming materials containing only $CaCO_3$ (Fig. 1).

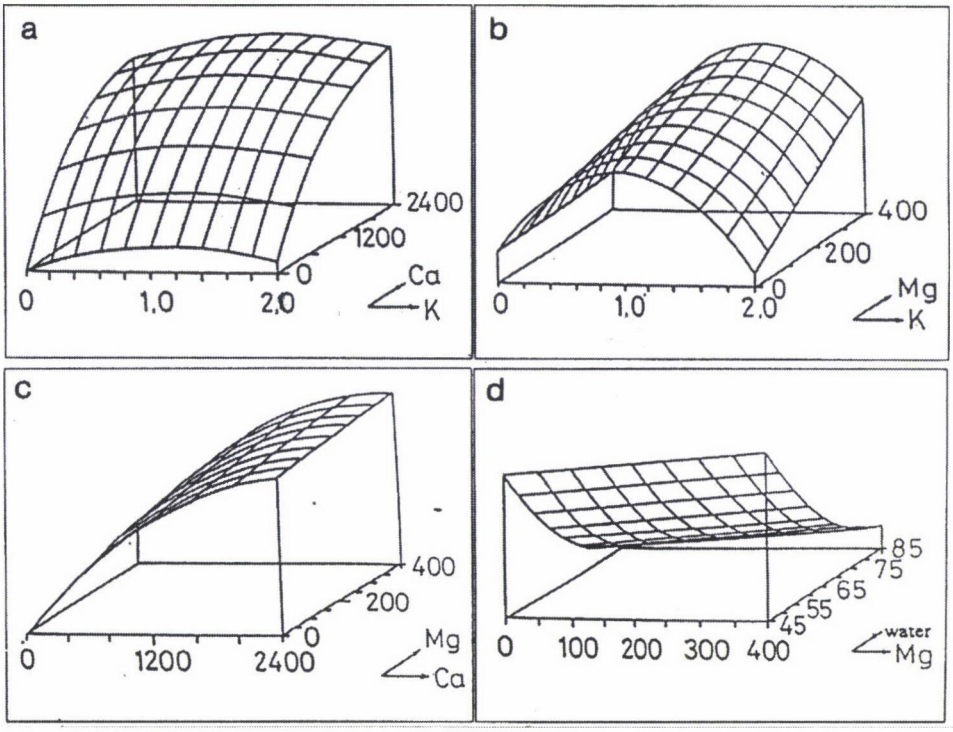


Fig. 1.a. Effect of potassium and calcium supplies on the grain yield at optimum magnesium and water supplies. Potassium increased the grain yield to a small extent, whereas calcium increased it significantly; *1.b.* Effect of potassium and magnesium supplies on the grain yield at optimum calcium and water supplies. Up to the optimum rate (1.2 g) potassium increased the yield; above this value, however, reduced it. The yield-promoting effect of magnesium increased as the potassium dose increased, i.e. the efficiency of magnesium is better at higher potassium doses; *1.c.* Effect of calcium and magnesium supplies on the grain yield at optimum potassium and water supplies. With optimum potassium and water supplies, calcium produced a significant and magnesium a slight increase in the yield. *1.d.* Effect of magnesium and water supplies on the grain yield at optimum potassium and calcium supplies. Water supplies exceeding the optimum level reduced the yield and inhibited the efficiency of magnesium

The favourable impacts of chemical amendments with different Ca:Mg ratios can be measured through changes in the equivalent element ratios of K/Ca + Mg in the crops. Liming materials that contain higher amounts of Mg are suitable for changing the unfavourable cation ratio in crops. With liming materials that contain only $CaCO_3$, the K/Ca + Mg ratio in the crops cannot be reduced to any great extent due to the increased Ca uptake, resulting in a decrease in Mg content (Balogh and Nyiri, 1982).

In acidic soils with a Mg shortage, toxic Mn uptake by the plants can also be related to unsatisfactory Mg supplies to the plants, within the given reaction state and redox conditions of the soil (Balogh, 1984).

Chemical amendments containing Ca and Mg can be used more efficiently than liming to mitigate the extremely high Mn uptake of plants in acidic soils with a Mg shortage. The availability of the Mg component of self-crumbling dolomites with an amorphous crystal structure is more favourable than that of ground dolomites. Therefore, instead of the expensive, energy-consuming heat treatment of dolomites, the use of natural self-crumbling dolomites seems to be more favourable (Balogh, 1988) (Fig. 2).

In the above-mentioned soils, joint Ca and Mg liming not only has a yield-increasing effect on certain crops (potato, sugarbeet, winter wheat, sunflower, spring barley), but also improves a number of quality traits [starch content, α-amino-N content, baking (SDS) value, oil content, thousand seed weight].

The effective duration of a full dose of dolomite, calculated on the basis of soil analysis data, is 6–8 years. The yield increase obtained by conventional liming was 0.35–0.42 t/ha WWE, but a surplus yield of 0.52–1.09 t/ha WWE could be achieved, averaged over the investigated years and crops with different Ca and Mg demands, with the use of self-crumbling dolomites (Fig. 3).

Among the crops investigated potato, maize and sunflower produced excellent increases in yield. Among the recent chemical amendments, dolomites with an amorphic crystal structure can be used most effectively to provide durable Ca and Mg supplies to the soil and to stop soil acidification. The chemical activity of these dolomites is almost as good as that of heat-treated forms (Balogh, 1992).

Main topics of Mg research on farm animals

The principal objectives of Mg research in this field were to improve the utilization of feeds and the productivity, fertility and health status of farm animals, and to study the role of Mg in biological and biochemical processes.

In practice, all these investigations serve the well-being of humans.

Magnesium and mineral supplementation and metabolism of various species

In exact experiments the metabolism – input and output – and the Ca, Mg and P requirements were studied in calves of different ages. It was found that fodder rich in Ca increased the amount of Mg excreted in faeces and urine, while P-rich fodder decreased it. The antagonism existing between Ca, Mg and P was proved by these findings. It was ascertained that calves are particularly sensitive to Mg deficiency (Urbányi, 1957).

Magnesium supplies and the performance and fertility of dairy cows, heifers, pigs and broilers

Highly productive new breeds and hybrids of high genetic value have now been developed, the performance of which can be improved with certain minerals, such as Mg.

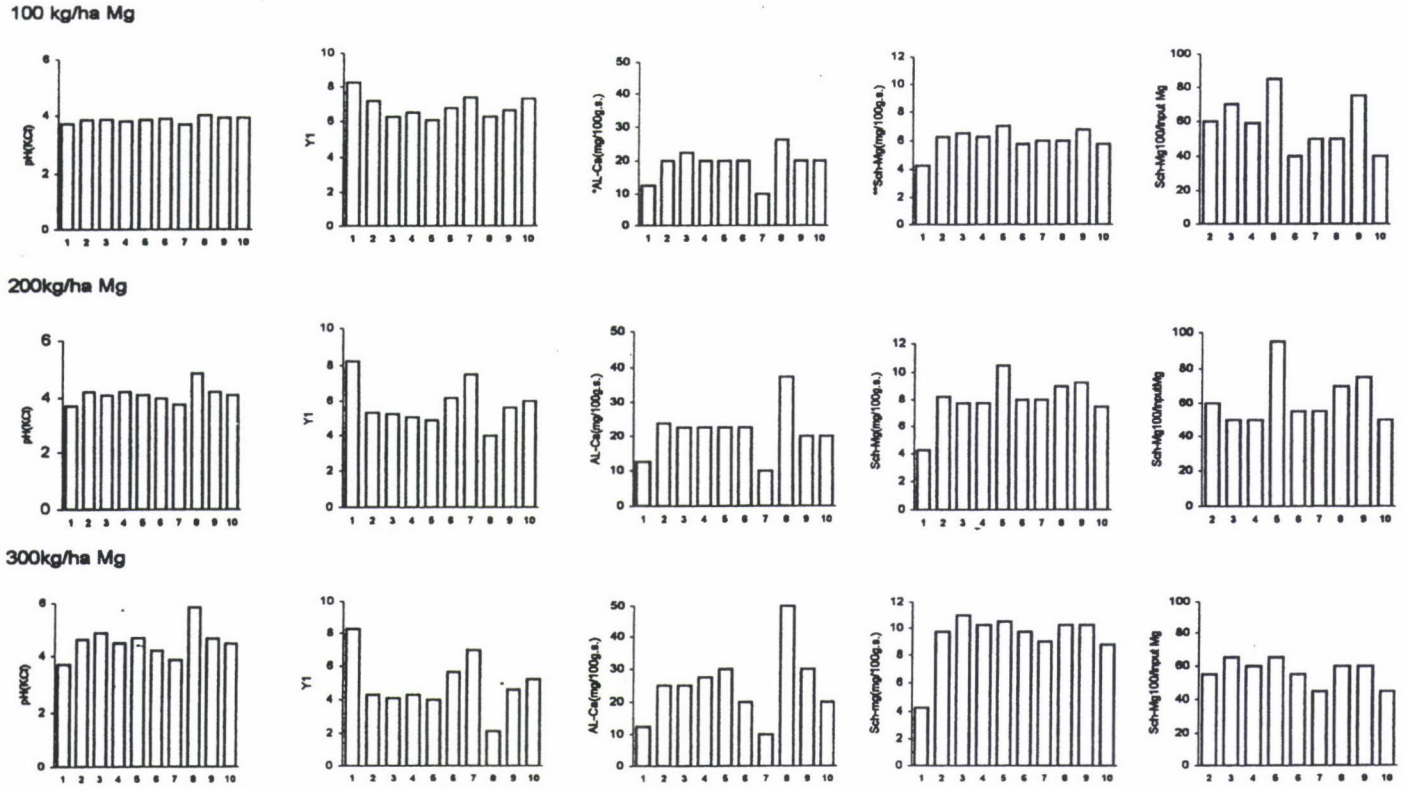
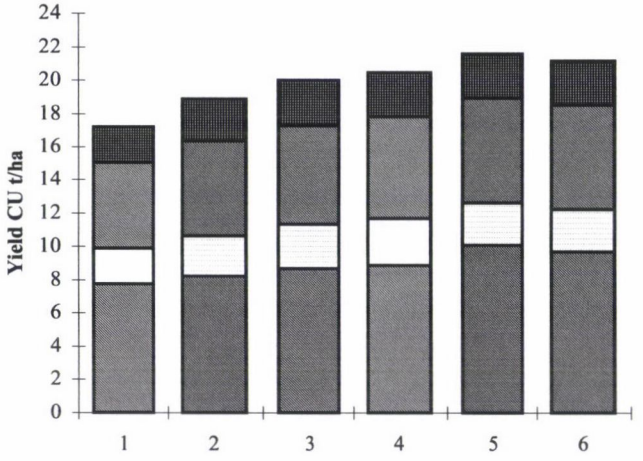
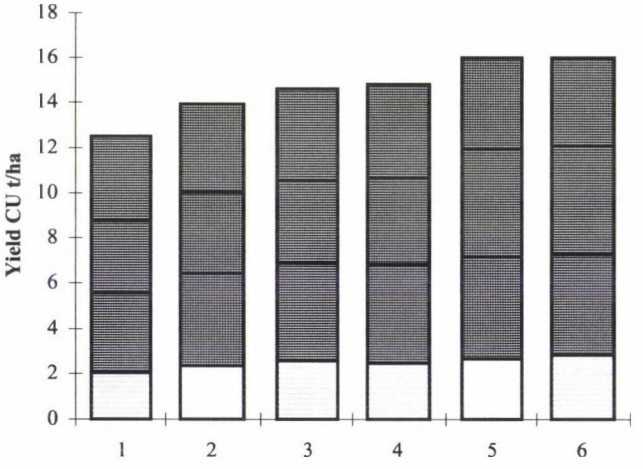


Fig. 2. Effect of various magnesium additives on the chemical properties of magnesium-deficient soil in a model pot experiment
Legends: 1. untreated, 2. heat-treated dolomite (700°C, 30 min.), 3. heat-treated dolomite (750°C, 60 min.), 4. heat-treated dolomite (780°C, 30 min.), 5. heat-treated dolomite (780°C, 60 min.), 6. heat-treated dolomite (780°C, 120 min.), 7. $MgCO_3.H_2O$ (nesquehonile), 8. lime from magnesite production, 9. self-pulverizing dolomite, 10. ground dolomite (*AL=ammonium lactate soluble, **Sch-Mg=Mg determined by the Schlachschlebel method)

Group A



Group B



Legends: potato sunflower maize horse-bean

1. Untreated, 2. Lime powder, 3. Lime powder Ca:Mg=3:1, 4. Lime from magnesite production Ca:Mg=3:1, 5. Self-powdering dolomite Ca:Mg=1.7:1, 6. Self-powdering dolomite Ca:Mg=1:1

Fig. 3. Effect of liming and ameliorative Ca-Mg replacement on crop yields

It was found that milk yields could be increased with Mg supplementation. The reproductive biological indexes of cows, heifers and pigs were also improved considerably by Mg supplementations to their basic diet (Kovácsné Gaál et al., 1987; 1993; 1997).

Magnesium status and metabolic disorders in ruminants

The disease grass tetany, or hypomagnesemic tetany occurs most frequently during the first weeks of the grazing season. In 1972, the magnesium status of a number of dairy farms was tested by measuring the concentration of Mg in the urine. Mg deficiency was found in only a few cases that year. However, the application of fertilizers rich in K and N might be a factor in increasing the incidence of hypomagnesemic symptoms, especially in young cattle (Tölgyesi et al., 1972).

Magnesium in the food chain (soil – plants – animals – foods of animal origin)

In a series of investigations the concentration of Mg was determined, among other things, in alfalfa, one of the most important plants in animal nutrition, harvested from different soils. It was found (Regiusné and Szentmihályi, 1975a) that the Mg content of a plant species depends significantly on the type of soil (Fig. 4).

As a result of another experiment it was concluded that the Mg content of the same plant species cultivated on the same type of soil also depended on the season of harvesting, i.e. on the age of the plants (Regiusné and Szentmihályi, 1975b). The Mg content increased in all species grown on the same soil from spring to autumn.

The Mg status of dairy cows was studied as a function of the Mg content of plants cultivated on different types of soil. The Mg level was found to be double the average in peas and sunflowers grown on swampy areas (Szentmihályi, 1962).

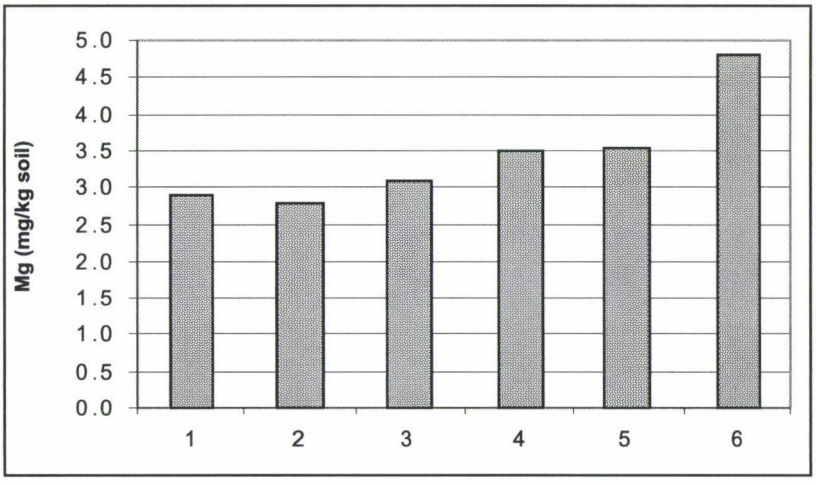


Fig. 4. Magnesium content (mg/kg) of alfalfa harvested from different soils. 1. Sand, 2. Bog, 3. Meadow-alluvial, 4. Sandy chernozem, 5. Chernozem-brown forest soil, 6. Salt-affected soil (Regiusné and Szentmihályi, 1975a)

Concentration of Mg and other minerals in various parts of the hoof horn related to species, ages, feedstuffs and different diseases

The consequences of large-scale, industrial, unnatural animal keeping systems were studied.

A high incidence of pulvinus formation on the hoof in swine can be observed under unsatisfactory conditions. This symptom is called "ragged hoof". Significantly higher concentrations of Mg and other minerals were found in these cases than in healthy animals, probably due to strong blood circulation, a chronic inflammation and a defensive response of the organism (B. Kovács and Szilágyi, 1974b).

The concentration of Mg was analysed in various anatomical regions of the hoofhorn in cattle, sheep and swine of different breeds, ages and keeping conditions. Differences in the concentration of Mg in anatomically and functionally different parts of the foothorn were found to be greater than the inter-breed differences between identical regions. The nails of piglets contained a much greater amount of minerals than those of adult swine (B. Kovács and Szilágyi, 1972a, b, 1974a).

Magnesium and other minerals in bones connected with mechanical properties

The strength of the skeleton is also affected by the keeping conditions. A close correlation was found between the structure, microhardness and rate of mineralisation (including the Mg concentration) of the bones and the keeping conditions (Szilágyi et al., 1978; 1980).

Effects of Mg supplementation on physiological features in wild animals

It was observed that the tusks of wild boars were fragile in certain areas of Hungary, causing financial losses to hunters. Treating these areas with Mg-rich dolomite proved to be effective in improving the quality of the tusks (Kiss, 1977).

Interactions between magnesium and certain trace elements in various species

A significantly lower concentration of magnesium was found in chickens exposed to lead and selenium (Table 2) and in rabbits exposed to lead, mercury and selenium (Table 3) than in the control (Szilágyi et al., 1995; 1996a, b). Interaction may exist between Cd and Se, as well as between Mg and Pb, Hg and Se. The decreased serum Mg levels might be due to the inhibited absorption and/or accelerated excretion of Mg caused by Pb, Hg and Se.

Table 2
Concentration of magnesium (mg/l) in the sera of chickens exposed to cadmium (0, 50, 100 mg/kg), lead (200, 400 mg/kg) and selenium (0.33 mg/kg)

Group	Mg (mg/l)
Control	24.5±1.8
Cd 50	23.2±1.3
Cd 100	23.3±2.5
Pb 200	22.7±2.4
Pb 400	21.5±1.3[a]
Se 0.33	20.6±1.6[b]

Significant differences between control and treated groups:
a = P<0.01
b = P<0.001

Table 3
Concentration of magnesium (mg/l) in the sera of rabbits exposed to lead, selenium, mercury, cadmium and molybdenum

Group	Daily intake (mg/animal)	Mg (mg/l)
Control	–	25.1±3.3
Pb	0.24	19.9±1.9[a]
Se	1.35	19.0±2.9[a]
Hg	0.12	17.2±4.1[b]
Cd	0.10	26.1±1.3
Mo	1.40	28.5±2.0

Significant differences between control and treated groups:
a = P<0.05
b = P<0.01

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Review

A REVIEW OF DECISION SUPPORT SYSTEMS FOR FERTILISER APPLICATION AND MANURE MANAGEMENT

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There are a number of Decision Support Systems (DSS) that provide advice on the application of inorganic fertilisers and organic manures to farmers. Here, seven of the most commonly used DSS (EMA, FERTIPLAN, MANNER, N-CYCLE, PrecisioN Plan, SUNDIAL-FRS and WELL-N) have been reviewed in detail, with regard to their capabilities, input requirements and flexibility. We have also compared these DSS to a commonly used UK reference book-based approach (RB209). No one DSS covers all crop types or all of the forms of organic wastes as well as inorganic fertilisers. We conclude that a) where possible, DSS should be based on dynamic models and b) further DSS development is needed, particularly for organic wastes, grassland systems, fruits and hops.

Key words: fertiliser application, manure management, decision support, review

Introduction

A number of personal computer-based decision support systems (DSS) have been developed to help farmers to minimise nitrogen (N) losses through leaching whilst maintaining an economic crop yield. The fertiliser recommendation systems (FRS) estimate crop N requirement, either at an average economic optimum yield or at the yield specified by the user. The supply of N from the soil, inputs from soil amendments, and losses from the system are then calculated, allowing an estimate of the optimum fertiliser application rate. This review focuses on DSS for the fertiliser application and manure management most commonly used in the UK.

A fully comprehensive FRS needs to be capable of making recommendations for the application of inorganic and organic fertilisers to a wide range of crops and agricultural systems. We first list the most commonly used DSS and then give a brief overview and introduction to each. The DSS capabilities are detailed with respect to the UK MAFF reference book (MAFF, 1994; hereafter referred to as RB209); input requirements are discussed and finally conclusions are made, based on the evidence presented.

Overview of Decision Support Systems

Table 1 provides an overview of the available DSS. A distinction must be made between dynamic and static model types. Dynamic models use experimentally derived equations to predict the transformation of nitrogen in the soil, and simulate these processes through time. Dynamic models are therefore able to respond to changing environmental conditions. Static models contain no temporal element, using a simple balance sheet approach with various modifications for local weather and soil type.

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Table 1
Overview of Decision Support Systems

Decision Support System	Model		Hardware requirements	Operating systems			Does the model account for		
	Dynamic	Static		DOS	Windows 3.1	Windows 95	Soil type/ texture?	Local weather?	Cropping history?
EMA		✓	IBM PC, Win 3.1 or above, 2Mb free disk space		✓	✓	✓		✓
FERTIPLAN		✓	IBM PC, small amount of disk space	✓			✓		✓
MANNER		✓	IBM PC, small amount of disk space		✓	✓	✓	✓	
N-CYCLE		✓	IBM PC, Win 3.1 or above, 2Mb free disk space	✓			✓	✓	✓
PrecisioN Plan		✓	IBM PC, 4Mb (Win 3.11) or 8Mb (Win 95) free disk space		✓	✓	✓	✓	✓
SUNDIAL-FRS	✓		IBM PC 486 or above, 5Mb free disk space		✓	✓	✓	✓	✓
WELL-N	✓		IBM PC, DOS v.3 or above, 1.54Mb free disk space	✓			✓	✓	✓

Table 2
Fertilisers and manures accounted for in the Decision Support Systems

Decision Support System	Inorganic N	FYM				Slurries				Sewage sludge				
		Cattle	Pig	Poultry	Weeping wall cattle	Dairy	Pig	Mechanically separated cattle	Broiler /turkey	Strainer Box cattle	Liquid undigested	Liquid digested	Cake digested	Cake undiges-ted
EMA	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
FERTIPLAN	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
MANNER		✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
N-CYCLE	✓	✓												
PrecisioN Plan	✓	✓	✓	✓	(✓)	(✓)	(✓)	(✓)	✓	(✓)	(✓)	(✓)	(✓)	(✓)
SUNDIAL-FRS	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
WELL-N	✓													

(✓) = Does not differentiate between different type

EMA

Environmental Management for Agriculture (EMA; Lewis et al., 1997) is a general computer-based toolkit to support good environmental practice in farming. The fertiliser recommendation module uses the guidelines in MAFF RB209 to make recommendations for inorganic fertilisers, covering arable, grassland and horticultural agro-ecosystems. Local soil texture and cropping history are accounted for.

FERTIPLAN

FERTIPLAN has been developed using historic field data, and is a static model-based DSS. FERTIPLAN makes recommendations for organic wastes and inorganic fertilisers, can be used for arable, grassland and horticultural crops, and accounts for local soil texture and cropping history. The system takes into account most of the organic waste types and crops in RB209.

MANNER

MANNER (MANure Nitrogen Evaluation Routine; ADAS, 1997) has been developed as a DSS for organic manures, and does not make recommendations for inorganic fertilisers. MANNER predicts the fate of N following organic manure applications to land. This DSS uses a static model for arable systems, and accounts for soil type and local weather, but not specific crops or cropping history.

N-CYCLE

N-CYCLE (Scholefield et al., 1991) calculates N transformations and losses from grazed grassland. It is based on a static simulation model developed from 10 long-term field grazing system experiments, and accounts for local soil texture, weather and cropping history. N-CYCLE can be operated for beef grazing, dairy cattle and cut-grass systems.

PrecisioN Plan

PrecisioN Plan (Hydro Agri, 1998) was developed from over 40 years of agronomic trials. It provides advice on the rate and timing of N for all major arable crops. This DSS uses a static (balance sheet) model to predict crop nitrogen requirements.

SUNDIAL-FRS

SUNDIAL-FRS (Smith et al., 1996; Smith et al., 1998; Glendining et al., 1998) is based on a dynamic N turnover model derived from the Rothamsted Nitrogen Turnover Model (Bradbury et al., 1993). This DSS will provide fertiliser recommendations for most arable, and some horticultural crops. It has been parameterised for all the common soil texture classes, except organic soils. SUNDIAL-FRS takes account of soil conditions, cropping history, manure applications and local weather data to provide a fertiliser recommendation specific to the current crop, field and season.

WELL-N

The WELL-N nitrogen advisory model (HRI, 1994) has been designed for growers and advisors, to enable a more accurate prediction of crop N fertiliser requirements. WELL-N is a dynamic model that can be used in arable and horticultural systems, but only makes recommendations for inorganic fertilisers.

Decision Support System capabilities

Fertilisers and manures

All the DSS reviewed, with the exception of MANNER, account for inorganic fertilisers (Table 2). EMA, FERTIPLAN, PrecisioN Plan and SUNDIAL-FRS account for a wide range of different organic manures and fertilisers, covering most of those included in RB209. MANNER estimates the amount of organic waste-derived available N remaining in the soil for the next crop. None of the reviewed DSS incorporate the nitrogen contribution made by dirty water or animal feeds.

In EMA, MANNER and SUNDIAL-FRS, it is possible to specify the composition of organic manures, but PrecisioN Plan only has one cattle slurry type, and simply liquid or cake sewage sludge. MANNER also includes fresh and old cattle and pig manures, plus layer manure and separated slurry solids, but not poultry manure.

Cropping systems

Table 3 shows the crops and systems accounted for in the reviewed DSS. All DSS, with the exception of N-CYCLE, can be used with arable crops. EMA, FERTIPLAN, PrecisioN Plan and SUNDIAL-FRS cover a wide range of arable crops (most of those in RB209), but WELL-N can make recommendations for one arable crop only. EMA contains data for field beans. FERTIPLAN includes winter and spring linseed. PrecisioN Plan includes winter linseed, spring linseed, and triticale and the system has a large range of different crop varieties; varieties are not featured in the other systems. MANNER does not include a description of the crop.

EMA, FERTIPLAN and SUNDIAL-FRS are the only DSS accounting for forage and cover crops. SUNDIAL-FRS accounts for four cover and forage crops, whilst EMA and FERTIPLAN can be used to make recommendations for all of the cover and forage crops listed in RB209.

EMA, FERTIPLAN, SUNDIAL-FRS and WELL-N all provide recommendations for horticultural crops, with the widest range of horticultural crops covered in EMA and FERTIPLAN. WELL-N includes Dutch, white and Chinese cabbage, as well as several types of lettuce, plus spinach and French beans.

Table 3
Crops and systems accounted for in the Decision Support Systems

Decision Support System	Arable crops	Forage crops	Horticultural crops	Root crops	Grain legumes	Grasslands	Fruits and hops
EMA	✓	✓	✓	✓	✓	✓	
FERTIPLAN	✓	✓	✓	✓	✓	✓	✓
MANNER	✓			✓	✓		
N-CYCLE						✓	
PrecisioN Plan	✓			✓	✓		
SUNDIAL-FRS	✓	✓	✓	✓	✓	✓	
WELL-N	✓		✓	✓	✓		

Table 4
Recommendations available and ease of use

Decision Support System	Application timing	Split applications	Economic optimum	Plant required optimum	Tracer simulations	Cycling/pool investigations	Whole Farm	Single Field	Ease of use		
									Easy	Moderate	Hard
EMA	✓	✓		✓				✓	✓		
FERTIPLAN	✓	✓	(✓)	(✓)				✓	✓		
MANNER						✓		✓	✓		
N-CYCLE						✓		✓	✓		
PrecisioN Plan	✓	✓	✓					✓	✓		
SUNDIAL-FRS	✓	✓		✓	✓		✓	✓	✓		
WELL-N	✓	✓		✓				✓		✓	

(✓) = Does not differentiate between different types

All systems except N-CYCLE and MANNER include root crops and grain legumes. PrecisioN Plan includes early and canning potatoes, but does not cover as many root crops and grain legumes as EMA, FERTIPLAN or SUNDIAL-FRS.

Only EMA, FERTIPLAN and N-CYCLE make recommendations for grassland systems, with EMA and FERTIPLAN covering all the systems in RB209. SUNDIAL-FRS takes account of the period under grass in the last 10 years in making recommendations for other crop types.

Only FERTIPLAN makes recommendations for fruit and hop crops.

Recommendations available and ease of use

Table 4 shows the recommendations available and ease of use of the reviewed DSS. All DSS except N-CYCLE provide information on the application timing, with the possibility of split applications. The nature of information provided by the DSS varies from economic optima to cycling and tracer studies, and the crop N requirement. All of the DSS, with the exception of WELL-N, are relatively easy to use. Only SUNDIAL-FRS is capable of producing whole-farm recommendations. In FERTIPLAN, recommendations for cereals are at the economic optimum, and for other crops, the crop N requirement is calculated. In N-CYCLE, SUNDIAL-FRS and WELL-N, the amount of N in different pools (fertiliser, atmosphere, volatilisation, denitrification, leaching, inorganic N, animal and plant uptake, dung, dead grass, beef, organic N, mineralisation) is calculated. MANNER and SUNDIAL-FRS predict the amount of N derived from organic wastes that will be available to the following crop.

Data requirements

Farm and field data

For all DSS, at least some farm and field data are required (Table 5). This can be input in the form of measurements (e.g. soil data) where it is known, or as default values derived from the farm location. In SUNDIAL-FRS, the farm location provides atmospheric N defaults, crop yield, sowing and harvest dates, and local weather. In N-CYCLE, soil texture and atmospheric N deposition are selected by location on a UK map. Other DSS (SUNDIAL-FRS, EMA, FERTIPLAN) provide default soil data by selecting a soil textural class. In MANNER, the user inputs a site name, plus the topsoil and subsoil texture class, which can be selected from a list.

Fertiliser and organic waste data

Most DSS require information about fertiliser and manure applications (Table 6), but in SUNDIAL-FRS and WELL-N, default values are available. In MANNER, default values for manure composition are available, but it is better to have an analysis.

Table 5
Data requirements – farm and field data

Decision Support System	Farm name	Location	Soil depth	Soil texture class	Soil nutrient data/analysis	Other soil information	Drainage	Period under grassland
EMA			E	E	D	Analysis desirable	D	D
FERTIPLAN	E	E	E	E	D	Analysis desirable		D
MANNER	E			E			D	
N-CYCLE				E			E	E
PrecisioN Plan	D	E	E	E	D	Analysis desirable		D
SUNDIAL-FRS	E	E	E	E	D	Atmospheric N desirable	E	E
WELL-N	D		D	E	D	Analysis desirable		D

E= Essential, D= Desirable

Table 6
Data requirements – fertiliser and organic wastes

Decision Support System	Inorganic Fertilisers			Organic wastes		
	Type	Application date	Amount	Type	Application date	Amount
EMA				E	E	E
FERTIPLAN	E	E	E	E	E	E
MANNER				E	E	E
N-CYCLE			E			
PrecisioN Plan	D		E	E	E	E
SUNDIAL-FRS	D	D	D	D	D	D
WELL-N	D	D	D			

E= Essential, D= Desirable

Crop and cultivation data

Most DSS require some cropping and cultivation data (Table 7), with the exception of MANNER. MANNER only requires the date of manure application, the delay before incorporation, and whether the manure was ploughed in within one month of application. Default input data are provided for use in SUNDIAL-FRS.

Weather data

Not all systems use or require weather data (Table 8). Some DSS can be used with local weather data where available, but default values can be set from the farm location (e.g. UK Ordnance Survey Grid Square, or county). In SUNDIAL-FRS, local weather data are generated internally from the location (selected from a list of counties), but the user can also input data manually or download from a data-logger or the local meteorological office station. In WELL-N, regional defaults are used to derive rainfall in the autumn and winter months. In PrecisioN Plan, a UK grid is used to select the location, which provides the summer and winter rainfall amount. In N-CYCLE, local weather data are determined by selecting a region from a UK map. MANNER uses the total rainfall from the date of application to the end of drainage. If this is not available, MANNER estimates a typical amount for the period between manure application and the end of drainage.

Summary and conclusions

We have reviewed the seven most commonly used UK DSS for fertiliser application and manure management, with regard to their capabilities, input requirements and flexibility, and compared them to a simple UK reference book-based approach (RB209).

No one DSS comprehensively covers all crop types, or all of the forms of organic wastes as well as inorganic fertilisers. All DSS require some form of input data, including site (soil, farm and field data), crop/cultivation, fertilisers/manures, and information on local weather. Default values for some of these inputs can be provided in the more advanced DSS. No DSS takes account of the contribution of dirty water or animal feeds. Only SUNDIAL-FRS and WELL-N are based on dynamic simulation models, incorporating a full response to changing environmental conditions. The other DSS are either simple balance sheets to calculate crop requirements or are based on RB209 itself, and are unable to account for year-to-year variability. This could prove a serious limitation in making informed estimates of crop fertiliser requirement, and so DSS based on dynamic models are recommended. However, dynamic model-based DSS may be limited by 1) data availability, and 2) the capabilities of the dynamic DSS.

Table 7
Data requirements – Crop and cultivation data

Decision Support System	Crops		Sowing date	Harvest date	Expected yield	Residue treatment	Irrigation		Cultivation		
	Current crop	Previous crop					Date	Amount	Date	Type	Depth
EMA	E	E									
FERTIPLAN	E	E	E	E	E	E					
MANNER									D	D	
N-CYCLE		E									
PrecisioN Plan	E	E	E							E	
SUNDIAL-FRS	D	D	D	D	D	D	D	D	D	D	D
WELL-N	E	E	E	E	E	E	D	D	D	D	D

E= Essential, D= Desirable

Table 8
Data requirements – weather data

Decision Support System	Timestep - day	Timestep - week	Timestep - month	Other timestep	Rainfall	Temperature	Other
EMA							Not used
FERTIPLAN							Not used
MANNER					D		
N-CYCLE							Defaults
PrecisioN Plan				Summer and winter rainfall			From UK Ordnance Survey Grid
SUNDIAL-FRS		✓			D	D	Evapotranspiration
WELL-N	✓				D	D	Evapotranspiration

E= Essential, D= Desirable

Of the 2 dynamic DSS reviewed, SUNDIAL-FRS is currently the most comprehensively developed, providing fertiliser recommendations for arable and horticultural crops and incorporating defaults for any unknown data inputs. However, SUNDIAL-FRS cannot provide recommendations for grassland systems or fruit and hops. MANNER, which is a static model, is the most appropriate DSS for organic manure recommendations. FERTIPLAN is the best-developed system for fruit and hops, which are currently not included in SUNDIAL-FRS. Further developments are needed to include grassland and fruit/hop systems in the dynamic models. Organic manure recommendations should also be incorporated in the future.

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Acta Agronomica Hungarica, 47(2), pp. 237–239 (1999)

Book reviews

G. P. RÉDEI: Genetics Manual. Current Theory, Concepts, Terms. 1998. World Scientific. Singapore, New Jersey, London, Hong Kong. 1142+IX pages. $78.00. ISBN: 981-02-2780-9

Although a number of dictionaries or glossaries of genetics already exist, this Genetics Manual is quite different from the others in-as-much as it is much more comprehensive, and not only defines the concepts but explains them in concise yet plain language. The book is a hybrid between an encyclopedia and a dictionary, and deals with about 18,000 concepts and terms. It includes 650 fairly simple, clear illustrations; many of them are suitable for drawing on a blackboard or projection onto a screen. The topics cover a broad field of genetics from the classical to the molecular, including many areas of cell biology and statistics. Some of the entries are just a single line, while others take up 5–6 pages. The majority of the statistical concepts are illustrated on fully worked-out examples and are therefore easy to use even for beginners. A particularly useful feature is the cross-referencing of the entries. Because of this, it is unnecessary to wade through a great mass of text to find the needed information exactly and quickly. It is also possible to read the book continuously by jumping from page to page according to the guided tour provided by the author and thus obtain as much information as needed. In the databases section, nearly 100 Internet addresses are provided and additional relevant databases are listed at the end of many entries. Some entries include specific references to key journal papers, and on pages 1117–1141 almost 1000 books – mainly published in the late 1990s – are listed according to their subject.

In a brief review it is impossible to enumerate all the topics discussed and of interest to students and researchers in agriculture, but I will mention a few to show the nature of this book. Mendelian laws, their cytogenetic bases and modified Mendelian ratios are presented in a historical and precise manner. Meiosis, mitosis and their differences are illustrated graphically. The manual includes the chromosome numbers of the most important organisms. Recombination in diploids and in autotetraploids and the molecular mechanisms involved in prokaryotes and eukaryotes are discussed. The use of the lod score is shown. The maximum likelihood principle and the underlying Bayes' theorem are logically expounded and shown in more detail than is to be found elsewhere. The procedures of recombinant DNA techniques, restriction enzymes, various cloning vectors and DNA sequencing are explained in sufficient depth.

Gene targeting systems such as *Cre/loxP* and *FLP/FRT* are illustrated and explained. Gene knockout, widely used now in animal genetics but missing from previous textbooks, is presented with clarity. Transcriptional and translational *in vivo* gene fusion vectors are explained. The essence of the transformation techniques is outlined for prokaryotes, fungi, plants and animals. Antisense technologies and some of the application potentials are pointed out. Gene therapy and cancer gene therapy are discussed. DNA chips and microarray hybridization are outlined.

Students interested in the quantitative aspects of genetics will be pleased with the step-by-step illustrations for calculating heritability, using the methods of intraclass correlation and regression. Fitness is explained by a lucid table. The computations used for evolutionary distance based on both proteins and DNA are exemplified. The bootstrap principle is shown. Paternity testing, association tests, ascertainment tests, the coefficient of coancestry and the inbreeding coefficient are explained in the simplest possible manner.

Immunogenetics, apoptosis, the cell cycle and many principles of cell biology are presented. Several pages are devoted to various aspects of mutation, and genetic loads, environmental mutagens, the fluctuation test, acquired characters, etc. are also covered. Mitochondrial and chloroplast genetics are discussed. About 200 human hereditary diseases are described, with chromosomal location and molecular mechanisms when known.

The Manual fills a void and I agree with other reviewers: "this book should prove very useful...for students, professionals and

non-professionals" (Quart. Rev. Biol. 74:74), "...invaluable to anyone..." (Acta Paediatr. 87:1211), "this manual is by far the best I have used" (HortScience 33:1274), "this book is very up-to-date" (Annals Int. Med. 130:168), "...an outstanding compendium of genetics" (Choice 36:1563, Nov. 1998).

J. SUTKA

Nyle C. BRADY and Ray R. WEIL: The Nature and Properties of Soils. 12th edition (1998) Prentice Hall, Upper Saddle River, New Jersey. ISBN: 0-13-852444-0

A fundamental knowledge of soil science is a prerequisite for meeting the many natural resource challenges that will face humanity in the 21st century.

The authors dedicated their book "... to all the students and colleagues in soil science who have shared their inspirations, camaraderie, and deep love of the Earth". In the Preface they expressed their opinion that soil provides an ideal system in which to observe practical applications for basic principles of biology, chemistry and physics and these principles can be used to minimize the degradation and destruction of one of the most important natural resources: *soil.* Consequently, the study of soils can be both fascinating and intellectually satisfying.

The publication of the twelfth edition marks the seventy-seventh year in which this classic book has helped inform and educate readers. It is the most widely adopted and read book on *soils* in the world. Hundreds of thousands of students in many countries have used it to launch their professional careers or merely to learn about soils as a *natural resource.*

In this new edition the main concept was to summarize the fundamental principles of soil science: to introduce soils, and to describe and explain their nature, physical, chemical and biological characteristics, formation and degradation processes (mass and energy regimes; transport, abiotic and biotic transformation) in a clearly understandable and richly illustrated manner. Particular emphasis was given to interactions between the soil and other components of forest, range, agricultural, wetland and constructed ecosystems. Environmental applications, management problems and human impact on soils were also discussed and presented.

The book includes *20 chapters*, as follows (the four numbers following the title are: number of pages; "boxes"; tables; figures):

1. The soils around us (28; 3; 2; 26).
2. Formation of soils from parent materials (42; 4; 1; 35).
3. Soil classification (46; 10; 1; 29).
4. Soil architecture and physical properties (54; 11; 5; 42).
5. Soil water: characteristics and behavior (42; 7; 3; 38).
6. Soil and the hydrologic cycle (52; 8; 0; 45).
7. Soil aeration and temperature (42; 7; 2; 31).
8. Soil colloids: their nature and practical significance (36; 8... 2; 21).
9. Soil reaction: acidity and alkalinity (35; 1; 4; 30).
10. Alkaline and salt-affected soils and their management (26; 7; 3; 15).
11. Organisms and ecology of the soil (42; 7; 1; 28).
12. Soil organic matter (45; 8; 4; 27).
13. Nitrogen and sulfur economy of soils (49; 13; 4; 30).
14. Soil phosphorus and potassium (45; 9; 2; 34).
15. Micronutrient elements (27; 7; 0; 19).
16. Practical nutrient management (56; 17; 2; 36).
17. Soil erosion and its control (55; 16; 1; 38).
18. Soils and chemical pollution (36; 15; 1; 18).
19. Geographic soils information (29; 4; 1; 18).
20. Global soil quality as affected by human activities (27; 16; 0; 14).

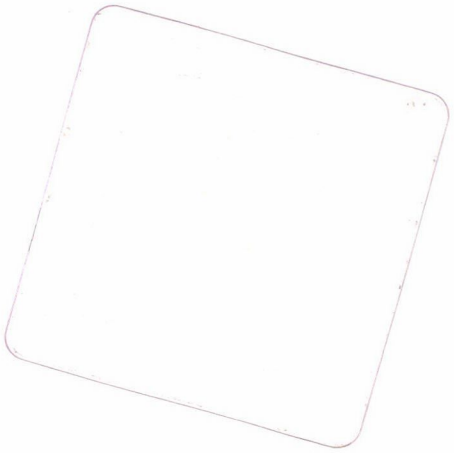
The content of the 20 thematic chapters is supplemented by *3 Appendixes* (A. U. S. Soil Taxonomy suborder map and legend; B. Canadian and FAO soil classification systems; C. SI unit conversion factors and periodic table of the elements); by a 35-page *Glossary* (containing the exact definition of 750 technical terms); and by an 18-page *Subject Index*. The clear index and glossary definitions are extremely helpful in teaching, in scientific discussions and

publications (to avoid misunderstandings and misinterpretation) and in the precise translation of English soil-related technical texts to other languages.

The main value of the book is the excellent and logical *structure*. The typography of the text makes the content clear, precise and easily understandable, with carefully and properly selected emphases. It is considerably helped by the 39 "boxes" that present either fascinating examples and applications or technical details and calculations. The 178 tables are informative, clear, not over-complicated and support the information, statements and conclusions in the text. The excellent illustrations, figures, photographs (altogether 575!) and the 35 colour plates represent unique value. All are selected well, constructed logically and prepared clearly, and their content is convincing and self-explanatory, making the whole book easily understandable: clear statements, convincing conclusions. The illustrations could be extremely useful in teaching, education and "popularisation" programmes in soil science and related subjects, giving useful and attractive guidelines for both students, teachers and university professors. The 1–2 page concise summaries (conclusions), 10–15 study questions, and the references (25–30 titles) at the end of each chapter represent considerable additional help in these activities.

The book is a huge and valuable information source, an excellent guideline and an exciting and enjoyable read for all "... who have shared their inspirations, camaraderie, and deep love of the Earth", as was expressed in the authors' introductory dedication. Let us hope that the book reaches a great number of specialists and "soil lovers" and successfully fulfils its "mission": to convince more and more members of human society of the particular significance of this multifunctional medium of natural resources: the *SOIL*.

G. VÁRALLYAY



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Ouyang, J. (1986): Induction of pollen plants in *Triticum aestivum.* In: Hu, M., Yang, M. (eds), *Haploids of higher plants* ***in vitro***. Academic Press, Beijing, 26–41.

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ORIGINAL PAPERS

SHORT COMMUNICATIONS

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RADIOSENSITIVITY OF GRAPEVINES. I. EMPIRICAL MODELLING OF THE RADIOSENSITIVITY OF SOME CLONES TO X-RAY IRRADIATION[+]

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Empirical ($1-[1-\exp^{-kD}]^n$) and formal (Poisson) models were utilized, applying experimental growth data to characterize the radiosensitivity of six grapevine clones to X-ray irradiation. According to the radiosensitivity constants (k), target numbers (n) and volumes, GR_{37} doses and energy deposition (nJ/μ^3), the following radiosensitivity order has been put forward: Chardonnay clone type < Hárslevelű K. 9 < Kövidinka K. 8 < Muscat Ottonel clone type < Irsai Olivér K. 11 < Cabernet Sauvignon E. 153. The model can be expanded to describe the radiosensitivity of other plant species and varieties, and also the efficiency of various radioprotecting agents and conditions.

Key words: *Vitis vinifera* L., ionizing irradiation, radiosensitivity, radioresistance, models

Abbreviations: Gy (Gray), 1 J energy absorbed by 1 kg plant material from ionizing irradiation; D, Dose (Gy); GR_{37}, dose for 37% growth reduction; k, radiosensitivity constant; n, number of target volumes; Y, growth reduction of dry matter in relation to non-irradiated plants

Introduction

In the physiological background of radiosensitivity/resistance the ploidy number of the chromosomes (Kothekar, 1989) and the hormonal (Arora et al., 1989; Jezierska-Szabó et al., 1987) and plant nutrition background play an outstanding role (Kőrösi, 1991).

Using plant height as a measure of radiosensitivity, 50% growth reduction (GR_{50}) was achieved with 148-169 Gy for *Pisum sativum*, 380 Gy for *Glycine max*, 506-582 Gy for *Centrosema pubescens*, 940-970 Gy for *Teramnus labialis*, 913-1357 Gy for *Stylosanthes humilis*, 997-1237 Gy for *Leucaena leucocephala* and 1086-1278 Gy for *Stylosanthes guianensis* (Perez-Talavera et al., 1989). Pollen, seeds and seedlings of *Phoenix dactylifera* were gamma-irradiated at different doses. LD_{50} (the dose at which 50% germinated/survived) was 0.2, 0.15 and 0.02 kGy for pollen, seeds and seedlings, respectively (Kgazal, 1989). Caryopses of 5 forms of *Triticum monococcum* and one of *Triticum sinskajae* were irradiated with 7 doses (20-220 Gy) of gamma rays and changes in various traits due to radiation were determined. The best index of radiation sensitivity was 9-day-old seedling length. Differences in response between the forms of *Triticum monococcum* were noted, with K6532 and K23653 reacting more

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markedly than the rest to low doses (60 Gy) but proving more radiation resistant at high doses (140-180 Gy), whereas the reverse pattern was seen in K35915. On the basis of germination percentage, *Triticum sinskajae* proved to be more sensitive than *Triticum monococcum* (Shcherbakov and Zolotova, 1989). The results of a preliminary study, to determine radiosensitivity in several pear cultivars, revealed that the OD_{50} (dose for 50% shoot growth reduction in comparison with controls) for the M_1 generation after the gamma irradiation of dormant buds was 4 krad in Hosui, Chojuro, Danbae and Deulbae and 6 krad in Shinsui and Niitaka. When active buds were irradiated the LD_{50} (dose for 50% death) was 3 krad in Hosui and 5 krad in Deulbae (Shin et al., 1988). Seeds of *Teramnus labialis* cv. Semilla Clara were irradiated with 0, 600, 800 or 900 Gy and sown. Seedling survival and height after 45 d were evaluated. Regression equations showed GR_{50} values of 511 Gy for survival and 584 Gy for plant height (Martin et al., 1989).

Seeds were treated with the radioprotector cystine and the rare earth element gadolinium before exposure to gamma rays at 50-500 Gy and thermal neutrons at $5 \cdot 10^{11}$ to $5 \cdot 10^{13}$ neutrons/cm^2. Radioresistance was measured by root growth and survival after treatment. In the case of neutron irradiation, cystine had no effect on radioresistance, while gadolinium increased it. The LD_{50} values for neutron and gamma radiation were positively correlated, and there was a negative correlation ($r = -0.74$) between the LD_{50} values and yield (Rashidov and Grodzinskii, 1991).

In the present work an attempt was made to elaborate models which would enable the radiosensitivity of the grapevines to be characterized and described more adequately than by the simple determination of LD, GR and OD dose response curves. A multihit (target) model to characterize the radiosensitivity of grapevines was set up. In the modelling the radiosensitivity constants, target numbers and volumes, GR_{37} doses and expected energy deposition in unit target volume were taken into account.

Materials and methods

Plant material

For the experiments single one-bud cuttings of clones of the following varieties and/or clones were used: Cabernet Sauvignon E.153, Chardonnay clone type, Hárslevelű, K. 9, Irsai Olivér K.11, Kövidinka K. 8, Muscat Ottonel clone type. Buds in dormant phenological stages were collected at the Research Institute for Viticulture and Enology, Kecskemét at the end of November. Using the results of an earlier bud radiosensitivity study on the varietal clones involved, the bud levels where the highest survival rate was observed (see Table 1) were included. After irradiation (see: irradiation conditions) the one-bud cuttings, with 10 replications for each clone and dose, were put in pots containing an appropriately moistened mixture of peat and perlite (3:1, v/v). The developed shoots were allowed to grow in a greenhouse at a temperature of 22 ± 2°C at 60-80% relative humidity until the controls reached the 4-5 leaf development stage, then the dry weights were measured and used for modelling.

Irradiation

For the irradiation a Liliput 140 X-ray apparatus (Medicor, Hungary) was used and the irradiation was performed at the Central Laboratory of the Agricultural University of Gödöllő. The doses applied were 10 Gy, 50 Gy, 100 Gy, 200 Gy and 400 Gy (120 kV, 4.5 mA).

Model used

A multihit (target) model to characterize radiosensitivity was set up using the function

$$Y = 1 - (1 - \exp^{-kD})^n \qquad (1)$$

where Y = growth reduction of dry matter in relation to the non-irradiated plant, k = the radiosensitivity constant, n = number of target volumes which is assumed in μ^3 and D = dose (Gy). The model for microorganisms, animal and human cells is discussed in detail in the literature (Fabrikant, 1972; Kudryasov and Berenfel'd, 1982; Weber, 1988). In order to calculate the radiosensitivity constants (k) and the target numbers, high dose approximation was applied (Weber, 1988) using the equation

$$Y = n \cdot \exp^{-kD} \qquad (2)$$

To fit the experimental data to these functions, an algorithm developed by Marquardt (1963) was employed. The procedure uses a search algorithm in an attempt to determine the estimates which minimize the residual sum of squares. This is essentially a compromise between using a straight linearization method and the method of the steepest descent. Then the growth reduction dose that reduces the dry matter production to any value between 0 and 1 was calculated according to the numerical solution of equation (1), that gives

$$D = -\frac{1}{k} \ln\left(1 - \sqrt[n]{1 - y}\right) \qquad (3)$$

In fact a growth reduction to 37% (GR_{37}) was assessed in order to presume a target volume, which was calculated by the equation

$$1/GR_{37} \qquad (4)$$

according to Kudryasov and Berenfel'd (1982). The expected energy deposition from the X-ray irradiation was estimated using GR_{37} as assessed by equation (3) and the calculated radiotarget volumes equation (4), postulating that the density of the sensitive volume was 1. For the calculation of deposited energy, the site approach was assumed, according to Kiefer and Kost (1988), Kőrösi et al. (1988) and Kőrösi (1991). The number of hits (j) from the doses used was estimated by kD. The Poisson probability distribution of the hits (Weber, 1988) was approximated according to the equation

$$P(j) = \exp^{-kD} \frac{(kD)^j}{j!} \qquad (5)$$

Results

The average X-ray GR_{37} dose which reduced the growth of the studied clones *via* dry matter production to 37%, was ≈ 166 ± 112 Gy. The range between the minimum and maximum GR_{37} doses amounted to 35 ÷ 356 Gy.

The calculated average target volume was ≈ 10 ± 9 μ^3. The mean energy deposition from the GR_{37} dose was ≈ 38 ± 46 nJ/μ^3 with a range of ≈ 125 nJ/μ^3. If ≈ 115 eV energy consumption is postulated to produce a three ion pair cluster (Fabrikant, 1972) an average ≈ $2 \cdot 10^9$ clusters was produced in 1 μ^3 target volume of the clones.

The adjusted radiosensitivity constant of Cabernet Sauvignon E.153 surpassed that of the Chardonnay clone type ≈ 5 times, and the assumed target volume ≈ 10 times (Table 1). For Cabernet Sauvignon E.153 ≈ 100 times less energy deposition in the unit target volume was needed to bring about a 37% growth reduction than for the Chardonnay clone type. Even the second radiosensitive clone (Irsai Olivér K. 11), according to our model, was given ≈ 24-fold more expected energy deposition for a 37% growth reduction in comparison with Cabernet Sauvignon E. 153. Thus, Cabernet Sauvignon E.153 appeared to be the most radiosensitive, with the most resistant being the Chardonnay clone type (Fig. 1).

The calculated number of hits from the applied doses is presented in Fig. 2. As can be seen, with the 300 Gy dose the most radiosensitive Cabernet Sauvignon E.153 received ≈ 8 hits, whereas the most resistant Chardonnay clone type only ≈ 1.5 hits.

The most radiosensitive clone, Cabernet Sauvignon E. 153, and the most radioresistant Chardonnay clone type (Figs 3 and 4) were compared in relation to the Poisson probability distribution of hits. Cabernet Sauvignon E. 153 received 10 hits/target volume at slightly more than 10% probability level and 4 hits at the 20% probability level. According to the model, the radioresistant Chardonnay clone type did not receive more than 2.2 hits/target volume (Fig. 4).

Table 1
Modelled characterization of radiosensitivity in grapevine clones

Variety/clone	Bud level	Fitted target No. ± std error	Fitted radiosensitivity const. ± std error	GR_{37} (Gy)	Assumed target volume (μ^3)	Assumed energy deposition at GR_{37} (nJ/μ^3)
Cabernet Sauvignon E.153	10th	0.9595 ± 0.0639	0.0271 ± 0.0056	35.44	28.22	1.26
Kövidinka K.8	8th	1.9559 ± 0.9630	0.0090 ± 0.0033	173.20	5.75	30.12
Irsai Olivér K.11	10th	1.8224 ± 0.2202	0.0188 ± 0.0055	79.59	12.56	6.33
Muscat Ottonel clone type	9th	1.0454 ± 0.0679	0.0071 ± 0.0029	144.98	6.90	21.01
Chardonnay clone type	10th	2.8099 ± 0.5290	0.0053 ± 0.0022	355.92	2.81	126.66
Hárslevelű K.9	7th	12.5540 ± 0.5372	0.0161 ± 0.0004	206.24	4.85	42.52

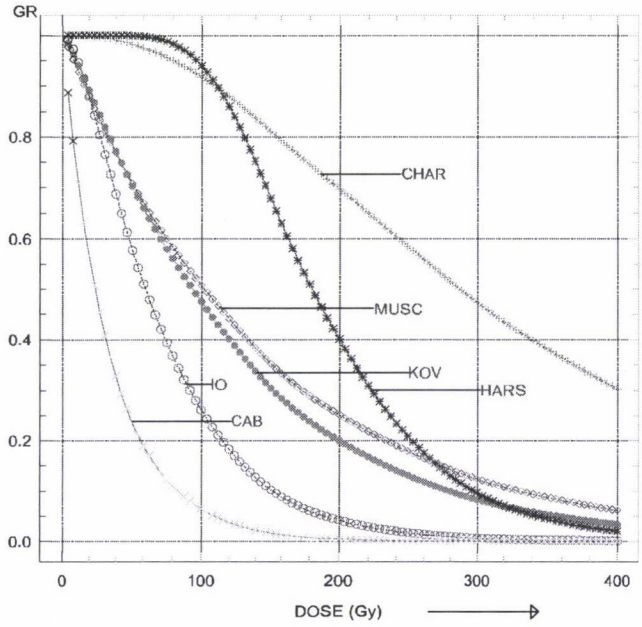

Fig. 1. Modelled radiosensitivity of the studied vine varieties/clones (MUSC = Muscat Ottonel clone type, KOV = Kövidinka K.8, IO = Irsai Olivér K.11, CAB = Cabernet Sauvignon E.153, HARS = Hárslevelű K. 9, CHAR = Chardonnay clone type)

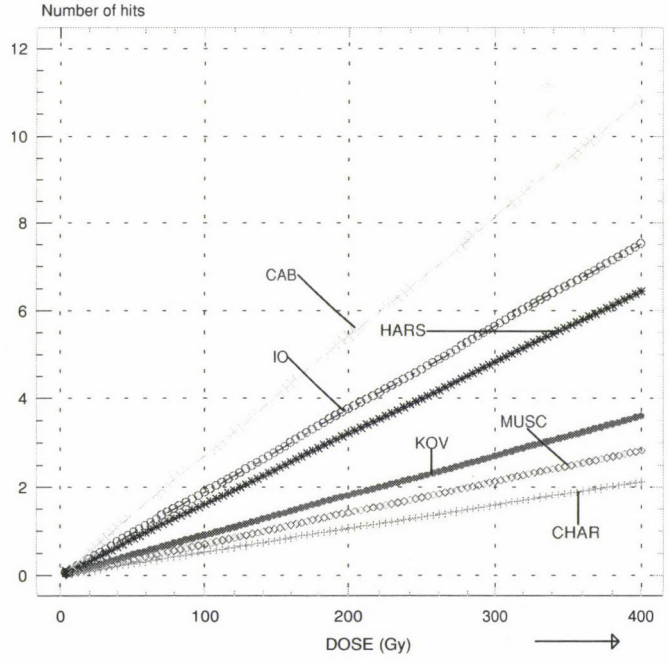

Fig. 2. Calculated hits for the investigated vine varieties/clones. Labels as in Fig. 1

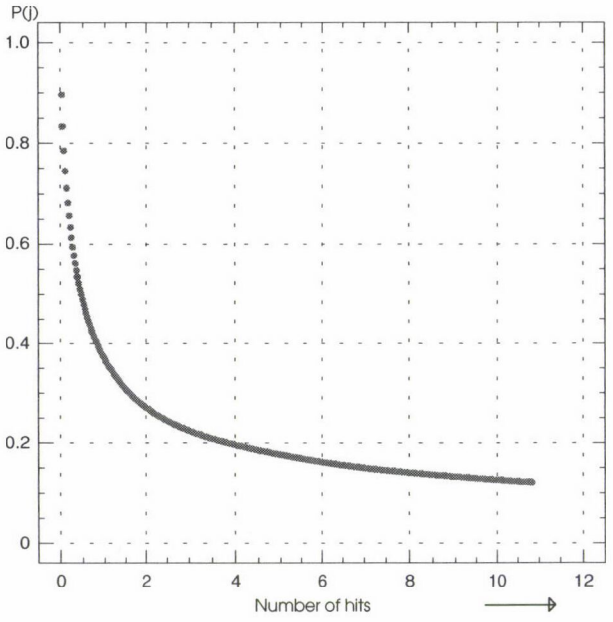

Fig. 3. Poisson probability distribution of hits for Cabernet Sauvignon E.153

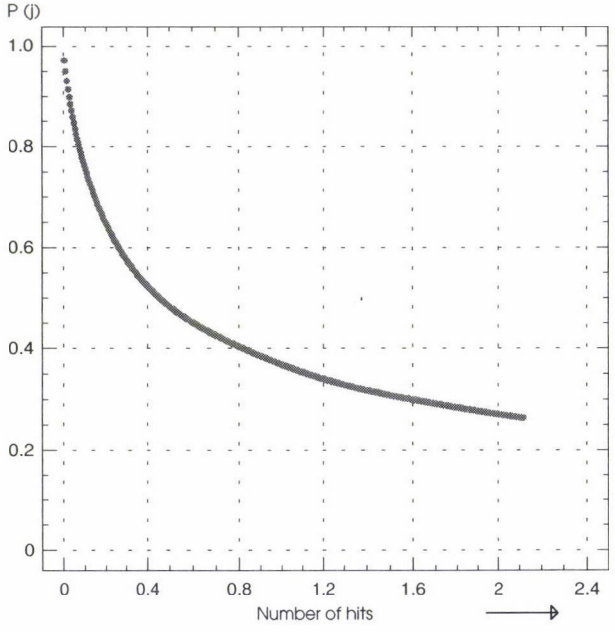

Fig. 4. Poisson probability distribution of hits for the Chardonnay clone type

Discussion

It is well documented in the literature that different plant species, different varieties, races and strains within each species, and organs, tissues, cells and enzymes show different degrees of radiosensitivity (Kőrösi and Pál, 1987; Maul et al., 1987; Kgazal, 1989; Kőrösi et al., 1988; Feng et al., 1991), which also depend upon developmental stages and environmental conditions (Kőrösi, 1991; Geras'kin et al., 1996). This can be related not only to the genetic, physiological and environmental conditions (Wang et al., 1993; He et al., 1994; Bruggemann et al., 1996) but also to the absorbed dose distribution patterns in the plant material at tissue, cell and even cell organ levels (Kőrösi and András, 1986; Kőrösi et al., 1988; Kiefer and Kost, 1988). A determination of the radiosensitivity of plant species, cultivars and clones is essential for assessing the repair and recovery capacity of the plants from radiation injuries (Hou et al., 1994; Joiner, 1994), for measuring factors influencing these (Elder and Osborne, 1993; Zhu et al., 1993) and for evaluating radioprotective agents (Aliev and Babaev, 1994).

In radiosensitivity determinations the dose effects in relation to survival, germination percentages, enzyme activities and banding patterns (Feng et al., 1991; Perez-Talavera et al., 1989), seedling and plant heights (Kothekar, 1989; Shcherbakov and Zolotova, 1989) and root and shoot growth (Shin et al., 1988) are most commonly measured.

As is clear from the survey outlined above, regression analysis has mainly been applied so far for the measurement of the radiosensitivity of plants (Martin et al., 1989; Rashidov and Grodzinskii, 1991) and LD_{50}, GR_{50}, OD_{50} dose values have usually been given. Appropriate models for characterizing the radiosensitivity of plants in general and of grapevines in particular are, therefore, lacking.

As is well demonstrated in Table 1, the radiosensitivity of the investigated clones can be characterized by the assumed target volumes, which are in the range 2.82÷28.22 μ^3. The lowest volume stood for the most radioresistant clone, the Chardonnay clone type, and the largest value belonged to the most sensitive Cabernet Sauvignon E. 153 clone type, while the in-between values indicated sensitivity orders. These values are of the same magnitude as for the cell nucleus volume and coincide well with the Sparrow et al. (1963; 1965) findings concerning the radiosensitivity of different species. After conducting experiments with gamma-rays and growing plants of many different species, they reached the conclusion that there was an inverse relationship between the radiation doses required to produce a certain degree of growth inhibition and the volume of the nucleus at interphase. This relationship meant that species having larger interphase nuclear volumes were more radiosensitive, suffering a certain extent of growth inhibition even at smaller doses. In the present model this meant 1.26 nJ/μ^3 for the most sensitive type, Cabernet Sauvignon, and 126.66 nJ/μ^3 for the most resistant clone type, Chardonnay, a 100-fold greater energy deposition. This conclusion can further be expanded by the findings of Miller

and Sparrow (1964) who interpreted radiosensitivity in terms of interphase chromosome value (ICV).

For the clones studied the average X-ray dose to bring about a 37% growth reduction (GR_{37}) was ≈ 166 Gy, with an average target volume of ≈ 10 μ^3.

The shoulders that appeared on the curves for the Chardonnay clone type and Hárslevelű K. 9 (Fig. 1) may either indicate a very intensive reparation intensity or high accumulation capacity for sublethal damage. They can be directly related to the target numbers (Table 1). They may also indicate a possible dose range for biopositive effects of X-rays for these clones. Both the "radiostimulation" aspects of these effects for plants and their constraints are discussed in detail in the works of Simon and Bhattachariya (1977), Pozsár (1978) and Friedman (1986).

The number of hits the clones received from the doses were very different (Fig. 2). A characteristic picture emerged if the hits were related to the target number: the most radioresistant Chardonnay clone type received ≈ 0.4 hits/target number from the 300 Gy dose, while the radiosensitive Cabernet Sauvignon E.153 received 8. Nevertheless, it is important to note that two hits were received at almost the same (20%) probability level by the radiosensitive and tolerant clones (Figs 3 and 4).

The radiosensitivity constants can also be used for assessing the effects of radioprotective agents, such as cystine, gadolinium, glutathione, ascorbic acid, plant growth regulators, etc. (Pozsár, 1978; Rashidov and Grodzinskii, 1991; Aliev and Babaev, 1994; Babaev et al., 1995).

For every clone the radiosensitive constants, GR_{37} doses, target volumes, number of hits and energy depositions were parameterized. Accordingly, the following order of radiosensitivity was postulated: Hárslevelű K 9 < Kövidinka K. 8 < Muscat Ottonel clone type < Irsai Olivér K. 11 < Cabernet Sauvignon E. 153. When radioresistance was considered the reverse order was found. When much more energetic gamma-rays were applied (1.13, 1.33 MeV *vs.* 120 KeV for the X-rays used here) varietal susceptibility was also observed in *in vitro*-derived *Vitis vinifera* L. plants, with the maximum dose withstood being 40 Gy for the Gravesac variety and 30 Gy for the Fercal variety. Shoot development was reduced more than root development and increasing doses led to a decreased rate of survival, rhizogenesis and growth of *in vitro*-derived plants (Lima-da-Silva and Doazan, 1995)

The results obtained can be used for radiomutational work, for nuclear biotechnology as well as in regions affected by isotope fallout. However, when assessing the radiosensitivity of the plants it should be considered that, according to the data gained from the zone exposed to 200–400 $\mu Gy \cdot h^{-1}$ after the Chernobyl accident, seeds from undamaged plants were the heaviest and had the highest germination energy and germinating ability, and were also more resistant to further gamma irradiation (Yushkov et al., 1994; Pozolotina, 1966). There is strong evidence for the existence of a cellular radioprotective mechanism that can be up-regulated in response to exposure to small doses of ionizing radiation. Either of these "induced" mechanisms protects against a subsequent exposure to

radiation that may be substantially larger than the initial "priming" dose. Work at molecular level is now confirming that changes in levels of some cytoplasmic and nuclear proteins, and the increased expressions of some genes, may occur within a few hours or even minutes of irradiation (Joiner, 1994). This would be sufficiently quick to explain the phenomenon of induced radioresistance, although the precise mechanism, whether by repair, cell cycle control or some other process, remains as yet undefined.

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A STUDY OF MAGNESIUM UPTAKE USING SZLOVÁK-TYPE WEIGHING LYSIMETERS

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Mg uptake was studied in weighing lysimeters in a 60,000 plants per hectare plant stand. Five maize hybrids were used in the experiment. At the end of the growth season the plants were harvested and the roots were washed out of the soil. After harvest the plant parts were separated and dried until constant weight was achieved. The plant parts were analysed for Mg using an atomic absorption spectrophotometer following digestion with sulphuric acid and hydrogen peroxide.

There were between 6 and 47% differences in dry matter weight and between 4 and 35% differences in transpiration between the values of various hybrids and the highest yield and transpiration of the given hybrids.

The highest Mg concentration in the whole plants was observed for the hybrid Sabrina 3707 and the highest total Mg content for hybrid Pi RSC 3732.

In all the hybrids examined the stem had the highest Mg concentration, and among the hybrids Sabrina 3707 showed the highest value (0.23%). As for the Mg concentration, the total Mg content was also highest in the stems of Sabrina 3707 (0.34 g).

The best physiological efficiency of Mg was obtained with Pi RSC 3732. There were no significant differences between the hybrids examined when the transpired water was calculated per unit weight of absorbed Mg and when Mg uptake was calculated per unit weight of transpired water.

The highest correlation between root mass and the Mg content of the whole plant ($r = 0.99^{*}$) was calculated for the hybrid Pi 3906. When the mean value of five maize hybrids was considered, the correlation was weaker ($r = 0.71^{***}$), but because of the larger number of data pairs it was significant at the P=0.1% level.

The best correlation between transpiration and Mg uptake was calculated for the same hybrid as in the previous case, that is for Pi 3906 ($r = 0.99^{**}$). For the mean value of the five maize hybrids the correlation again decreased ($r= 0.88^{***}$), but the level of significance increased.

While there was no significant difference between the K/Mg ratio of the whole plants, the K/Mg ratio of the leaf-blades and grain was significant.

Key words: maize genotypes, transpiration, magnesium uptake, K/Mg ratio

Introduction

Magnesium is absorbed by roots from the soil. It moves passively in the transpiration stream in an acropetal direction in the plant. Magnesium is found in plants in many forms: in free ionic form, in proteins, in nucleic acids and bound to anionic groups of phospholipids and in chlorophyll. The magnesium in chlorophyll makes up only 15–20% of the total Mg in the plant (Pethő, 1993). The smallest magnesium deficiency occurs at pH 5. At higher values Mg uptake is limited by Ca ions, and at lower values by H ions (Mengel, 1961). Mg is the central atom of the chlorophyll molecule and cannot be functionally substituted by any other atom.

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The Mg absorbed is dissolved in the cells and, similarly to K and Ca, influences the swelling and the colloidal state of the protoplasm. In the seeds there is always more Mg than Ca. Mg plays an important role in storage materials. According to Dunn and Roberts (1954) Mg can be absorbed even if it is in the form of organic salt.

Rainy years may cause Mg deficiency. Mg deficiency may occur not only because of washing out by water, but also by the fact that in wet summers the ions of monovalent elements (K and Na) are more mobile than the bivalent Mg and thus limit Mg absorption (Kiss, 1983).

In nutrient solution containing cadmium the root and shoot growth of rice seedlings was retarded. This could be counterbalanced by magnesium application, but only if the magnesium concentration was a hundred times higher than the cadmium concentration (Oncsik et al., 1989).

Magnesium uptake is continuous during the growing season (Kreutz, 1977).

Since Mg plays an important role in green plants, promoting P uptake, and since various maize genotypes show significant differences in Mg accumulation (Gallaher et al., 1981) the weighing lysimeter experiment was set up using 5 maize hybrids to examine the Mg uptake at optimum water supplies.

Materials and methods

In the experiment 150–200 kg rider scales were installed deep enough in the soil for the upper edge of the weighing lysimeters to be level with the surrounding soil surface. The plate iron frame saved the scales from caving in. On the area surrounding the lysimeters a plant density corresponding to 60,000 plants/ha was ensured.

Each lysimeter was filled with air dry alluvial meadow soil from the surface horizon (in the region of the town of Szarvas) corresponding to 85 kg of absolutely dry soil. Other characteristics of the soil used in the experiment: pH_{H_2O}: 6.7, pH_{KCl}: 5.7, total salt %: 0.06, humus %: 2.06, total N %: 0.14. Available P and K, determined by the methods outlined by Egner et al. (1960), were: P: 36.3 and K: 179.4 ppm.

The amount of fertilizers applied per lysimeter was: N: 3.5 g (ammonium nitrate), elemental P: 0.61 g (superphosphate), elemental K: 1.44 g (KCl).

Five maize hybrids were examined in the experiment (Szentesi 212, Pi 3907, Pi SC 3902, Pi RSC 3732, Sabrina 3707). Five maize seeds of each hybrid were sown per lysimeter and after emergence the plants were thinned to one. There were four replications. For the transpiration measurements the lysimeter soil was covered with PVC film; thus, the water loss from the lysimeter was due solely to transpiration by the plants. The lysimeter soil was irrigated to 70% of its maximum water-holding capacity and watering took place when 30–35% of the plant available water was lost by transpiration. Thus the plants grew under optimum soil moisture conditions. The transpiration was measured daily.

At harvest the soil was washed from the roots and after separation, the plant parts were dried at 60°C until constant weight. The plant parts were analysed for Mg by atomic absorption spectrophotometry following digestion with sulphuric acid and hydrogen peroxide.

Results and discussion

Water utilization

All five maize hybrids transpired most during the pentad between 22 and 27 July (Fig. 1). Depending upon the hybrids, during these five days the plants transpired between 10.7 and 11.6% of their total transpiration during the growth season. A slightly lower transpiration peak was observed in all the hybrids in the pentad between 11 and 16 August. The transpiration peaks coincided with the maximum values of air temperature.

The total dry matter weight, including roots, is presented in Table 1. The hybrid Pi 3906 gave the lowest dry matter yield and Pi RSC 3732 the highest. The lowest and highest values of transpiration were also observed for these hybrids. Transpiration coefficients were calculated for the whole plant, aboveground part and grain. Though the hybrid Pi RSC 3732 transpired the most, the transpiration coefficients of this hybrid, calculated for the whole plants, aboveground part and grain, were more favourable than for the other hybrids because of the high dry matter yield. The transpiration coefficients of Sabrina 3707 were unfavourable because of its low dry matter yield. The transpiration coefficient calculated for the grain was especially low. This can be explained by the longer growth season of Sabrina 3707; late sowing did not permit full development.

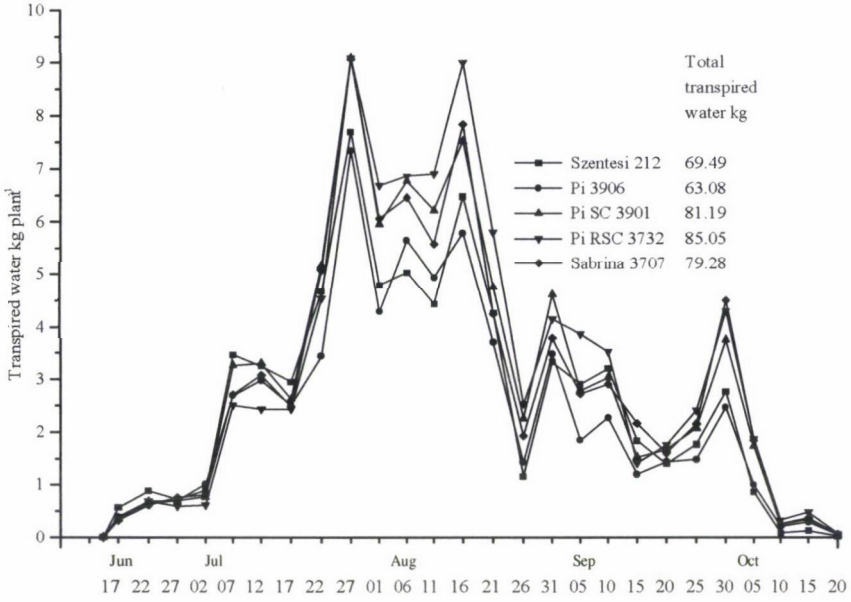
Concentration and total Mg in whole plants

Figure 2 illustrates the dry matter yield, Mg concentration and total Mg content of the maize hybrids. The dry matter yield of the hybrids Pi RSC 3732 and Pi SC 3901 was significantly higher than that of the other three. For Pi RSC 3732 this difference was significant at the 0.1% level.

The Mg concentration in the whole plant of the hybrid Sabrina 3707 was the highest (0.15%), while the smallest values were obtained for the hybrids Pi 3906, Pi SC 3901 and Pi RSC 3732. The percentage Mg in these hybrids was the same (0.12%). The Mg concentration of Szentesi 212 was 0.14%. The Mg% of Sabrina 3707 was significantly higher at the 5% level than that of the hybrids Pi 3906, Pi Sc 3901 and Pi RSC 3732. Similar magnesium percentages were obtained by Thomas et al. (1960; 0.11–0.22%) and Gorsline (1961; 0.11 and 0.21%) in field-grown maize inbreds and single-cross maize hybrids.

Among the hybrids examined Pi RSC 3732 showed the highest Mg content (0.76 g). The hybrid Pi 3906 contained the least Mg (0.51 g). Pi SC 3901, Pi RSC 3732 and Sabrina 3707 contained significantly more Mg than Pi 3906 at the 5% level.

The maize plants (with roots), calculated for 60,000 plants/ha, contained between 30.6 and 45.6 kg Mg depending on the hybrids. In a field experiment carried out by Lásztity et al. (1985) the maize plants contained between 19.4 and 36.9 kg Mg/ha.



Days after emergence	2	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	105	110	115	120	125
Szentesi 212	0.8	1.3	1.0	1.3	5.0	4.7	4.2	6.7	11.1	6.9	7.2	6.4	9.3	6.1	1.7	4.8	4.2	4.6	2.6	2.0	2.5	4.0	1.2	0.1	0.2	0.0 %
Pi 3906	0.6	1.0	1.1	1.6	4.3	4.7	4.0	5.4	11.6	6.8	8.9	7.8	9.2	5.9	2.3	5.5	2.9	3.6	1.9	2.3	2.3	3.9	1.6	0.3	0.5	0.1 %
Pi SC 3901	0.4	0.8	0.9	0.9	4.0	4.1	3.2	6.4	11.2	7.3	8.3	7.6	9.3	5.9	2.8	5.7	3.4	3.7	1.9	2.1	2.5	4.6	2.1	0.3	0.4	0.1 %
Pi RSC 3732	0.5	0.8	0.7	0.7	2.9	2.9	2.9	5.3	10.7	7.9	8.1	8.1	10.6	6.8	3.0	4.9	4.5	4.1	1.6	2.1	2.8	5.0	2.2	0.4	0.6	0.1 %
Sabrina 3707	0.4	0.8	0.9	1.0	3.4	3.9	3.1	6.4	11.4	7.6	8.1	7.0	9.9	5.3	2.4	4.8	3.4	3.7	2.7	2.0	2.7	5.7	2.3	0.3	0.5	0.1 %

Fig. 1. Percentage transpiration of maize hybrids over 5-day periods

Table 1
Transpiration coefficients of maize hybrids

Maize hybrids	Dry matter yield of whole plant (g)	Total transpired water (kg)	Transpiration coefficients		
			Whole plant	Aboveground part	Grain
Szentesi 212	435.26	69.49	159.54	175.71	453.60
Pi 3906	415.58	63.08	151.50	172.02	405.33
Pi SC 3901	574.75	81.19	141.64	164.71	330.27
Pi RSC 3732	612.18	85.05	139.08	157.72	299.55
Sabrina 3707	448.95	79.28	177.40	217.19	742.94
$LSD_{0.1\%}$	154.23	25.56	35.08	48.16	183.75
$LSD_{1.0\%}$	111.79	18.52	25.43	34.91	133.18
$LSD_{5.0\%}$	80.72	13.38	18.36	25.20	96.16

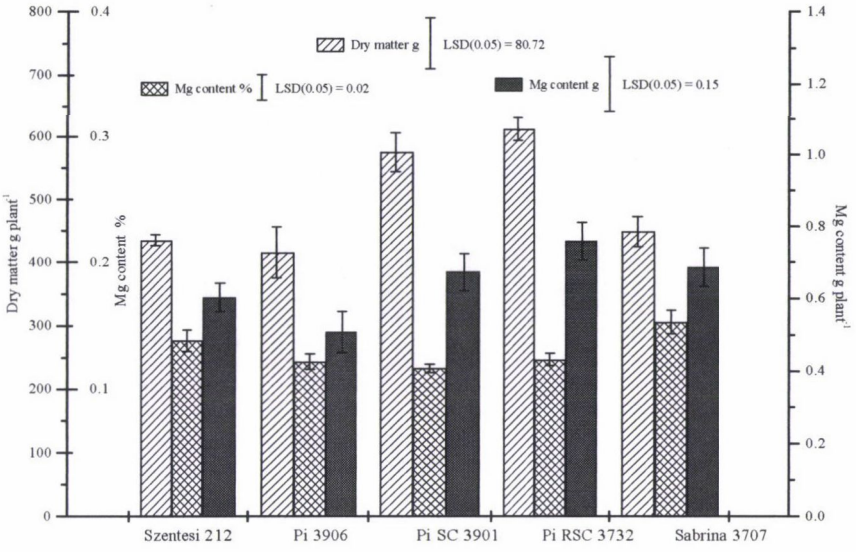


Fig. 2. Dry matter, Mg concentration and total Mg content of maize hybrids

Concentration and total Mg in plant parts

Figure 3 shows the dry matter yield, % Mg and total Mg content of the plant parts. The stem had the highest Mg concentration in all the hybrids examined. The highest value was obtained for Sabrina 3707 (0.23%), followed by Szentesi 212 and Pi RSC 3732 (0.20%). The smallest Mg concentration was exhibited by Pi SC 3901 (0.14%). This was significantly lower at the 0.1% level than the highest value. The second highest Mg concentration among the plant parts was found for the roots (0.16–0.19%), but there was no significant difference between them. The Mg concentration of the husks was higher than that of the leaf-blades. The lowest Mg percentage among the plant parts was found in the cob. The limit value of Mg concentration in the grain was between 0.08 and 0.11%.

Like the Mg concentration, the highest total Mg content was observed in the stem of Sabrina 3707 (0.34 g), while the least Mg was found in the grain in this hybrid (0.10 g). Among the plant parts the cob and the ear shank contained the least total Mg. While the roots were in second place as regards the Mg concentration, the second largest value of total Mg content was obtained in the grain. The highest Mg content in the grain was found for Pi SC 3901 (0.25 g), followed by the hybrid Pi RSC 3732 (0.24 g). The least Mg in the grain was recorded for the hybrid Sabrina 3707 (0.10 g).

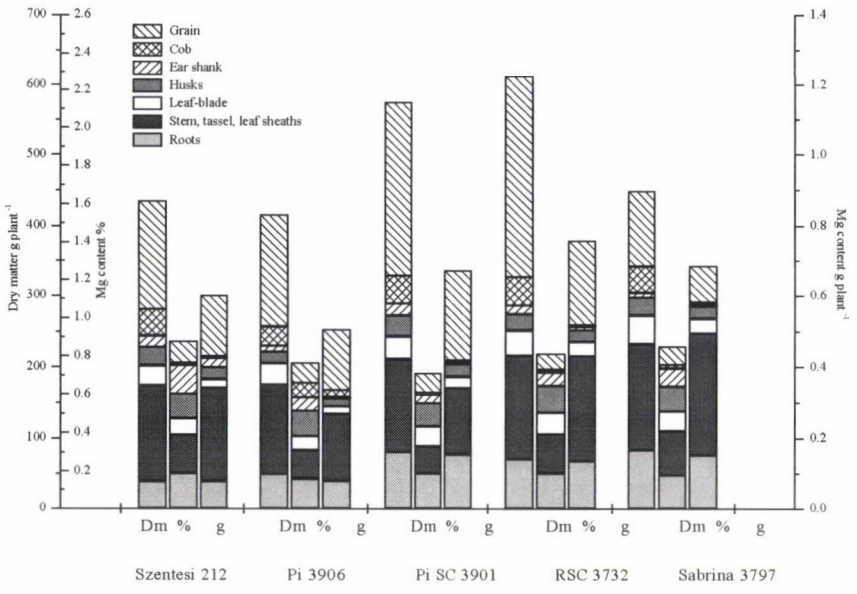


Fig. 3. Dry matter, percentage and total Mg content of plant parts

Dry matter yield calculated per unit mass of absorbed Mg

The greatest dry matter yield calculated for one g of absorbed Mg was produced by the hybrid Pi SC 3901 (Table 2). The smallest value was obtained for Sabrina 3707, which was only significantly lower than the highest value at the 1.0% level. There was a significant difference at the 5.0% level between Pi SC 3901 and Szentesi 212. The average value of the five maize hybrids was 778.71 g.

Table 2
Dry matter weight of whole plants (including roots) calculated per 1 g of absorbed Mg

Maize hybrids	Dry matter yield (g)	Total Mg (g)	Dry matter weight calculated for 1 g of absorbed Mg (g)
Szentesi 212	435.26	0.60	731.93
Pi 3906	415.58	0.51	826.63
Pi SC 3901	574.75	0.67	859.78
Pi RSC 3732	612.18	0.76	813.63
Sabrina 3707	448.95	0.69	661.57
$LSD_{0.1\%}$	154.23	0.29	215.31
$LSD_{1.0\%}$	111.79	0.21	156.06
$LSD_{5.0\%}$	80.72	0.15	112.68

Physiological efficiency of Mg

The best physiological efficiency of Mg, i.e. the grain yield calculated per unit weight of absorbed Mg, was obtained for Pi RSC 3732 (Table 3). The smallest grain yield calculated per unit weight of absorbed Mg was recorded for the hybrid Sabrina 3707, which was significantly lower at the 1.0% level than that of the other four hybrids. The hybrid Szentesi 212 was only significantly surpassed by two hybrids, Pi SC 3901 and Pi RSC 3732.

Transpiration water calculated for unit weight of absorbed Mg, and Mg uptake calculated for unit transpired water

The hybrid Pi 3906 transpired the most water (122 kg) per 1 g Mg absorbed (Table 4). The least transpired water (112 kg) was recorded for Pi RSC 3732. Though there were differences between the hybrids, none of them was significant. There was also no significant difference between the hybrids when Mg uptake was calculated for 1 kg of transpired water. The Mg uptake calculated for 1 kg of transpired water over the average of the five hybrids was 8.63 mg.

Table 3
Physiological efficiency of Mg

Maize hybrids	Grain mass (g)	Mg content of whole plant (g)	Grain mass calculated per 1 g of Mg (g)
Szentesi 212	154.18	0.60	260.98
Pi 3906	158.45	0.51	320.84
Pi SC 3901	245.85	0.67	369.93
Pi RSC 3732	285.08	0.76	378.16
Sabrina 3707	106.88	0.69	158.73
$LSD_{0.1\%}$	57.82	0.29	135.70
$LSD_{1.0\%}$	40.96	0.21	98.35
$LSD_{5.0\%}$	29.18	0.15	71.02

Table 4
Transpiration water calculated per unit of absorbed Mg and Mg uptake per unit of transpired water

Maize hybrids	Transpired water (kg)	Total Mg uptake (g)	Transpired water calculated per 1 g of absorbed Mg (kg)	Mg uptake per 1 kg of transpired water (mg)
Szentesi 212	69.49	0.60	113.69	8.83
Pi 3906	63.08	0.51	122.38	8.17
Pi SC 3901	81.19	0.67	120.25	8.37
Pi RSC 3732	85.05	0.76	112.08	9.04
Sabrina 3707	79.28	0.69	115.52	8.73
$LSD_{0.1\%}$	25.56	0.29	30.76	2.36
$LSD_{1.0\%}$	18.52	0.21	22.29	1.71
$LSD_{5.0\%}$	13.38	0.15	16.10	1.23

Correlation between root mass weight and the Mg content of the whole plant

The correlation coefficient was quite different for the various hybrids. The weakest correlation (r = 0.25) was calculated for the hybrid Pi RSC 3732, and the best correlation (r = 0.99*) for the hybrid Pi 3906 (Fig. 4). The mean value of the five hybrids exhibited a weaker correlation (r = 0.71***), but because of the larger number of data pairs it was significant at the 0.1% level (Fig. 5).

Transpiration and Mg uptake

When examining the correlation between transpired water and the total Mg content of the whole plant it can be seen that the lowest correlation was observed for the hybrid Pi RSC 3732 (r = 0.34) and the highest for the hybrid Pi 3906 (r = 0.99**, Fig. 6). The mean value of the five correlations decreased (r = 0.88***), but the level of significance increased (Fig. 7). The correlation between transpiration and the uptake of different elements by the whole plant was not the same in the individual hybrids. While in the case of phosphorus the lowest correlation was calculated for the hybrid Szentesi 212 (r = 0.43; Szlovák, 1995), the lowest correlation in the case of Mg was observed for the hybrid Pi RSC 3732.

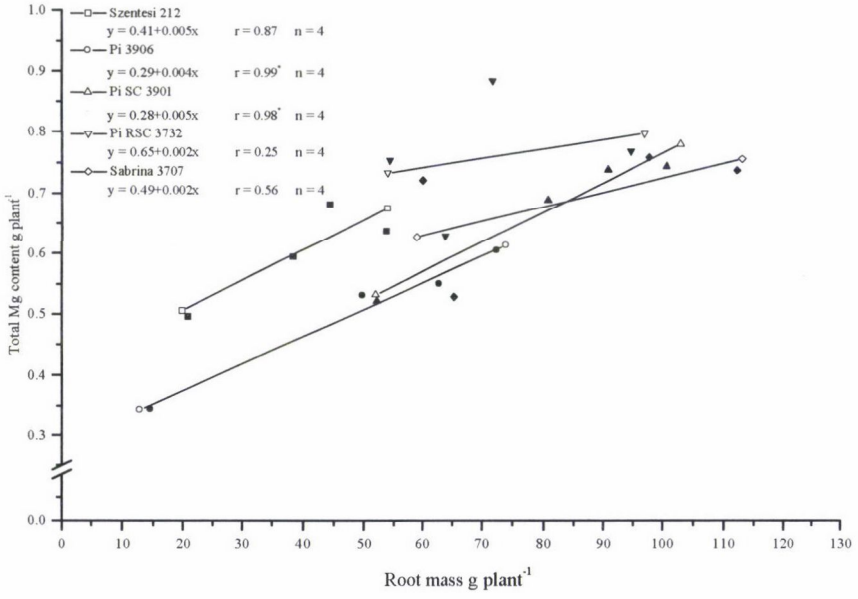


Fig. 4. Relationship between the dry matter yield of maize roots and the Mg content of the whole plant

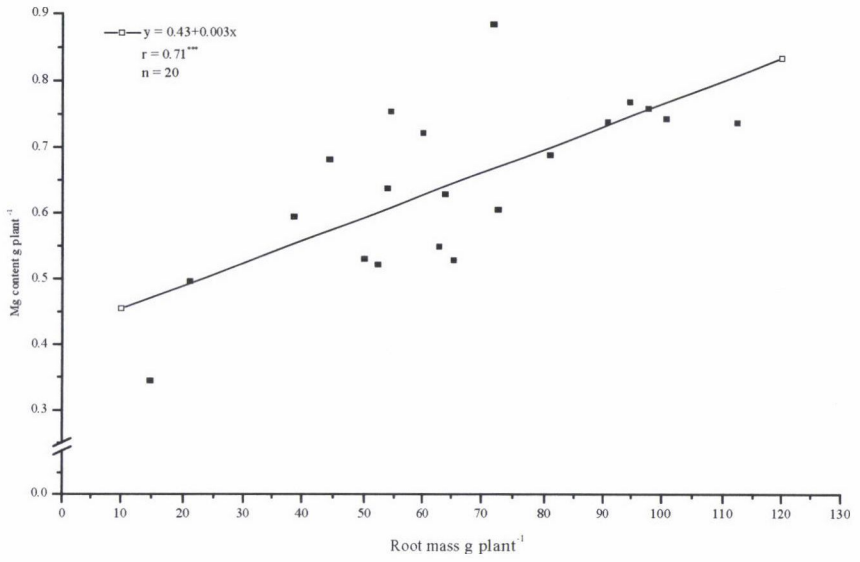


Fig. 5. Relationship between the root dry matter yield of five maize hybrids and the Mg content of the whole plant

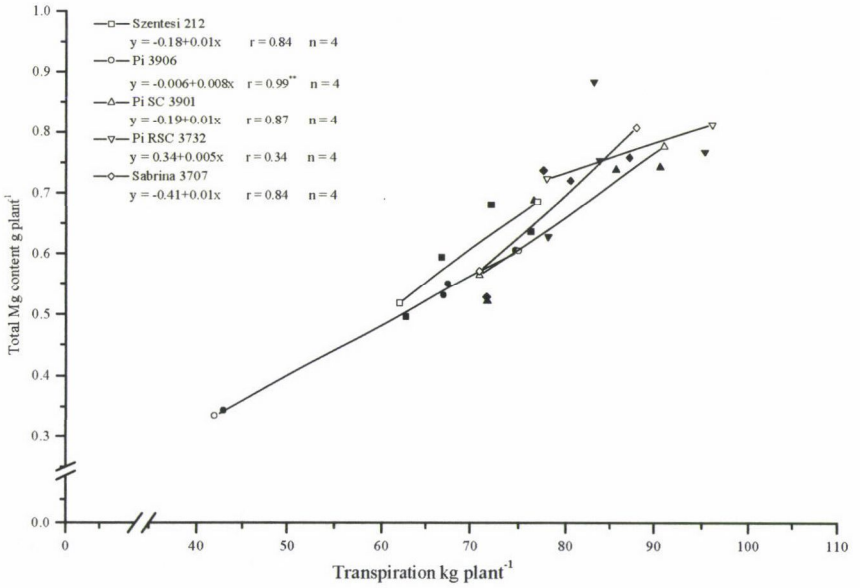


Fig. 6. Relationship between the transpiration and Mg content of the whole maize plant

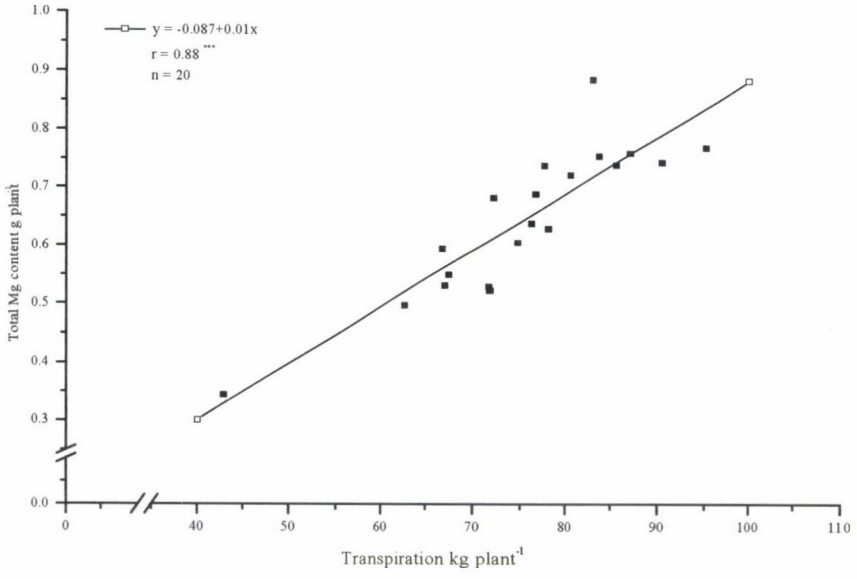


Fig. 7. Relationship between the transpiration of five maize hybrids and the Mg content of the whole plant

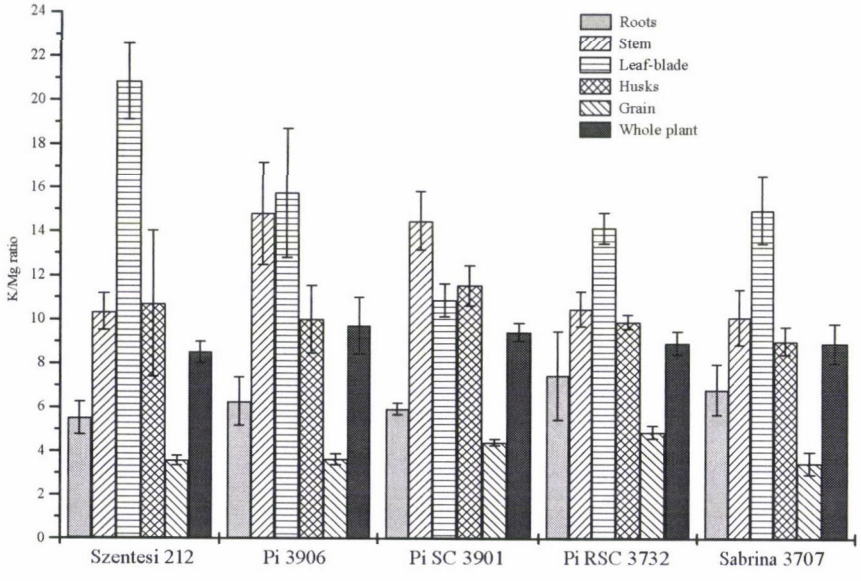


Fig. 8. K/Mg ratio of maize hybrids

K/Mg ratio

The calculation of the K/Mg ratio was made not only for the whole plants but also for the plant parts at the end of the growing season (Fig. 8). While there was no significant difference between the K/Mg ratio of the whole plants, the K/Mg ratio of the leaf-blades and grain was significant. The K/Mg ratio of the leaf-blades of the hybrid Szentesi 212 was significantly higher at the 5% level than in three other maize hybrids (Pi SC 3901, Pi RSC 3732, Sabrina 3707), while the K/Mg ratio of the grain was higher in Pi RSC 3732 than in three other maize hybrids (Szentesi 212, Pi 3906, Pi SC 3901), also at the 5% level of significance. In a field experiment carried out by Lásztity et al. (1985) on chernozem-like sandy soil the mean K/Mg ratio of the grain was lower (2.18) than that obtained in the present studies over the average of five maize hybrids (3.98).

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RESPONSE OF SEEDS OF *CICER ARIETINUM*, *LENS CULINARIS* AND *TRIGONELLA FOENUM-GRAECUM* TO THE INTERACTIVE EFFECT OF SALINITY AND THIAMINE OR ASCORBIC ACID

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Increasing NaCl concentrations significantly reduced the germination capacity, radicle and plumule lengths, and fresh and dry matter yields of *Cicer arietinum*, *Lens culinaris* and *Trigonella foenum-graecum* seeds, especially at high salinity levels (120 and 160 mM). On the other hand, free amino acids, including proline, progressively accumulated as the NaCl level increased.

Soluble proteins and carbohydrates in seedlings of the three experimental species remained more or less unchanged at the 40 mM NaCl level. However, at higher salinity levels (120 and 160 mM) in *Cicer arietinum* and *Lens culinaris* seedlings a decrease in carbohydrates was accompanied by an increase in soluble proteins, whereas in *Trigonella foenum-graecum* an opposite effect was obtained. The level of 40 mM NaCl had a pronounced stimulatory effect on all the variables studied.

Seed soaking presowing in thiamine or ascorbic acid (50 ppm) partially or completely counteracted the adverse effects of salinity on the germination, seedling growth and metabolic activities of *C. arietinum*, *L. culinaris* and *T. foenum-graecum* plants.

Key words: *Cicer arietinum*, *Lens culinaris*, *Trigonella foenum-graecum*, NaCl, thiamine, ascorbic acid

Introduction

Germination and seedling growth as well as some metabolic activities of certain glycophytic plants are known to be altered by salinity stress (Flowers et al., 1977; Ahmed et al., 1980; Heikal et al., 1982; Khan and Naqvi, 1984; Shaddad et al., 1990; Azooz, 1997). The degree of these alterations depends mainly on the plant growth stage and the plant type. Many crop species are also depressed by salinity; they display a wide range of sensitivity to salinity (Greenway and Munns, 1980). Depending on the species, different strategies occur which may lead to salt tolerance (Yeo and Flowers, 1986; Weimberg and Shannon, 1988). Shaddad et al. (1990) found that although broad bean and lupin plants were tolerant to approximately the same salinity levels, the strategy of osmotic adjustment differed in the two species. In broad bean the adjustment was mediated by soluble carbohydrates and proline, but in lupin only by soluble proteins.

Attempts were made to employ phytohormones, the amino acid proline or vitamins to overcome the drastic effects of salinity on seed germination and seedling growth (Heikal and Shaddad, 1982; Shaddad et al., 1990; Verma et al., 1991; Singh et al., 1994; Hamed and Al-Wakeel, 1995; Azooz, 1997).

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The soaking of seeds of responsive cultivars presowing with vitamins could thus be exploited to ensure better germination and enhanced early seedling growth, particularly under stress conditions in dryland farming (Khan and Ansari, 1984; Samiullah et al., 1985), to enhance the grain yield at harvest (Kudrev and Pandev, 1965). Vitamins of the B group, apart from acting as coenzymes, are involved in various physiological processes, e.g. niacin and thiamine have been shown to act as growth promoters (Thimann, 1965). Similarly, thiamine and pyridoxine produced by leaves are essential for the growth of roots (Greulach and Adams, 1963). Moreover, Oertli (1987) reported that under certain conditions the exogenous application of vitamins to plants stimulates their growth. Thus, apart from their main role as coenzymes, it is not improbable that vitamins may also play other independent roles in the biochemical processes of plants, repairing the injurious effects of unfavourable conditions.

Therefore, in this work experiments were carried out with thiamine and ascorbic acid in an attempt to alleviate the inhibitory effects of salinity on the germination, seedling growth and some metabolic activities of *Cicer arietinum*, *Lens culinaris* and *Trigonella foenum-graecum*. These plants are dominantly cultivated in Upper Egypt and could be cultivated in newly reclaimed arid areas.

Materials and methods

The *Cicer arietinum*, *Lens culinaris* and *Trigonella foenum-graecum* seeds used in the present investigation were obtained from the garden of the Agronomy Department, Faculty of Agriculture, Assiut University. The salt stresses used were 0.0 (Control), 40, 80, 120 and 160 mM NaCl in 1/10 Hoagland solution (Hoagland and Arnon, 1950). Seeds of the control group were germinated using only 1/10 Hoagland solution as a substrate. Preliminary screening for various concentrations (10 to 500 ppm) of thiamine or ascorbic acid was made to obtain the optimum response and a concentration of 50 ppm was selected. To evaluate the interactive effects of thiamine or ascorbic acid with salinity, seeds of the experimental plants were soaked presowing in either thiamine or ascorbic acid solution (50 ppm) for 4 hours, then air-dried for 48 hours.

Seed germination

Ten seeds of the test species were pretreated with 10% Clorox (5.25% sodium hypochlorite) for 4 min., washed three times with sterilized distilled water, and then germinated in Petri dishes (9 cm diameter) at about 25°C. Three replicates (Petri dishes) were prepared for each treatment. The seeds were considered to be germinated after the radicle emerged from the testa. Another experiment was carried out simultaneously to study the interaction between treatments with thiamine or ascorbic acid and salinity stress on seed germination. The final percentage of germination was recorded after a period of 4 days.

Seedling growth

Ten seeds were placed between folded paper towels, covered by plastic film, rolled up, and placed upright in 600 ml beakers. Eighty ml of solution were used to saturate the towels in each of the treatments, and the seeds were treated in the same manner as described for the germination experiments. The seeds were left to grow in darkness in an incubator at 25°C. Distilled water was added as needed to compensate for evaporation losses. At the end of the experimental period (10 days) the seedling plumule and radicle lengths were recorded in addition to fresh and dry matter yields.

For the determination of water-soluble carbohydrates, a known weight of powdered tissue was extracted with distilled water for two hours in a boiling water bath. After cooling, the extract was filtered and the filtrate was completed to a definite volume, after which the water-soluble carbohydrates were determined by the anthrone sulphuric acid method (Fales, 1951).

Free proline was determined in 10-day-old seedlings according to the method described by Bates et al. (1973). The total free amino acids, other than proline, were extracted from the plant tissues and determined according to the method of Moore and Stein (1948). The soluble protein fraction was determined according to Lowry et al. (1952).

The data were statistically analysed by one-way analysis of variance (PC-state computer program) and the least significant difference (LSD) was used to test the difference between treatments.

Results

The data presented in Figure 1 reveal that the germination rate of salt-stressed *Cicer arietinum*, *Lens culinaris* and *Trigonella foenum-graecum* plants remained more or less unchanged at the lowest salinization level used (40 mM NaCl). At above these levels the rates were significantly lowered. This inhibitory effect of relatively high levels of NaCl was more obvious in *Cicer arietinum* and *Lens culinaris* than in *Trigonella foenum-graecum.*

Treatment with thiamine or ascorbic acid (50 ppm) completely alleviated the inhibitory effects of NaCl salinity on the rate of germination of the experimental plants especially at low and moderate salinization levels. Thiamine exhibited a greater alleviative effect in the case of *Cicer arietinum* plants than ascorbic acid. In the case of *Lens culinaris* ascorbic acid was more effective than thiamine.

With respect to growth parameters, increasing salinity levels resulted in a significant reduction in the radicle and plumule lengths of the three test plants. This effect was more obvious at higher NaCl levels whatever the plant tested. However, in *Trigonella* plants a concentration of 40 mM NaCl had a non-significant effect on the lengths of the radicles and plumules. Treatment with vitamins (thiamine or ascorbic acid) resulted in a marked and progressive increase in the lengths of the three species of seedlings as compared with those of the corresponding untreated ones. This stimulatory effect was more pronounced in *Trigonella foenum-graecum* and *Lens culinaris* than in *Cicer arietinum* seedlings, whatever the type of vitamin added. It is worth mentioning that the effect of thiamine on the radicle lengths was more obvious than that of ascorbic acid. By contrast, treatment with ascorbic acid exerted a more obvious effect on the plumule lengths than thiamine, whatever species was tested (Table 1).

The fresh and dry matter yields of *Cicer arietinum*, *Lens culinaris* and *Trigonella foenum-graecum* seedlings remained more or less unchanged at the lowest NaCl level used (40 mM). There was a significant decrease in these values with rising salinity levels (Table 2). This reducing effect was more prominent at the highest stress level used (160 mM). Vitamin treatments considerably stimulated the production of fresh and dry matter in the seedlings of

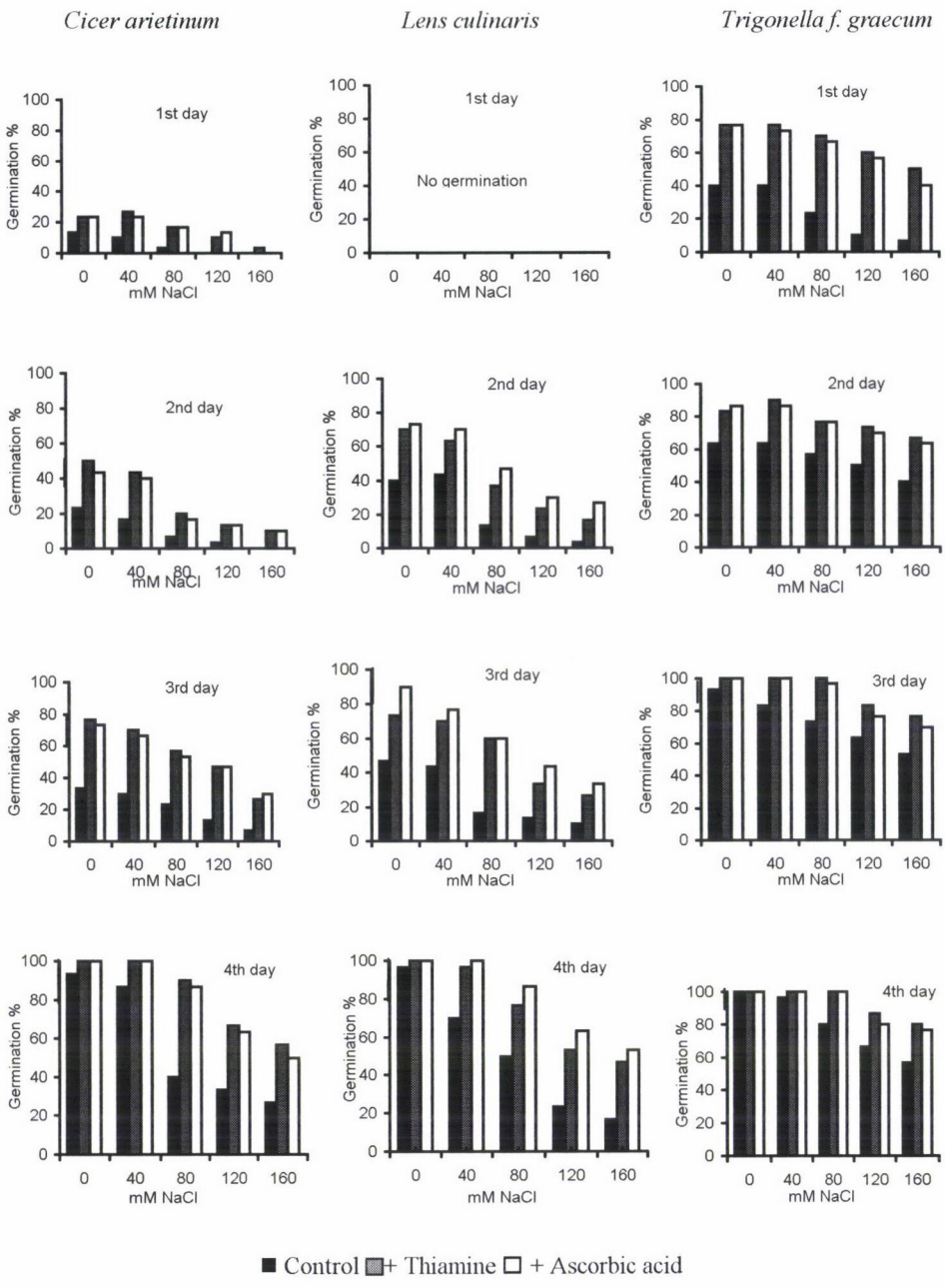

Fig. 1. Interactive effects of salinity-thiamine and salinity-ascorbic acid on germination percentage of *Cicer arietinum*, *Lens culinaris* and *Trigonella foenum graecum* seeds. Vertical lines indicate least significant differences ($P < 0.01$) for salinity and ascorbic acid or thiamine treatments

Table 1
Interactive effects of salinity and seed soaking in thiamine or ascorbic acid on radicle (R) and plumule (P) lengths (cm seedling^{-1}) of *Cicer arietinum, Lens culinaris* and *Trigonella foenum-graecum* seedlings

Treatments	NaCl (mM)	*Cicer arietinum*		*Lens culinaris*		*Trigonella f.graecum*	
		R. length	P. length	R. length	P. length	R. length	P. length
Reference control	00	12.1	6.1	9.60	6.30	4.90	7.75
	40	8.8	4.9	7.60	4.60	4.70	8.60
	80	5.1	3.2	4.50	3.80	3.00	7.30
	120	3.2	2.7	3.10	1.97	2.50	3.40
	160	2.5	1.9	2.60	1.50	1.30	2.40
+ Thiamine	0	13.8**	6.75	10.8*	7.80**	6.00**	8.80*
(50 ppm)	40	10.0**	5.9**	8.30	6.50**	5.80**	9.29
	80	6.3*	4.00*	6.40*	5.70**	4.60**	8.10
	120	4.9**	3.10	4.40**	2.50**	2.90	6.10**
	160	3.2	2.30	4.00**	2.20**	1.70	3.79**
+ Ascorbic acid	0	13.2*	7.63**	9.80**	8.30**	5.50*	9.10**
(50 ppm)	40	9.1	6.20**	7.90	7.20**	5.20*	9.60**
	80	6.2*	4.40**	6.00**	6.10**	4.00**	8.70**
	120	4.3*	3.20	4.00*	3.30**	2.70	7.10**
	160	2.9	2.66**	3.30	2.50**	1.70	4.70**
$LSD_{5\%}$		1.00	0.68	0.78	0.73	0.46	0.89
$LSD_{1\%}$		1.35	0.92	1.06	0.98	0.61	1.21

* Differences significant at the P = 0.05 level compared to the control
** Differences significant at the P = 0.01 level compared to the control

Table 2
Interactive effects of salinity and seed soaking in thiamine or ascorbic acid on fresh (F) and dry matter (D) yields (g seedling^{-1}) of *Cicer arietinum, Lens culinaris* and *Trigonella foenum-graecum* seedlings

Treatments	NaCl (mM)	*Cicer arietinum*		*Lens culinaris*		*Trigonella f.graecum*	
		F. weight	D.weight	F. weight	D.weight	F. weight	D.weight
Reference control	0	0.32	0.023	0.099	0.0065	0.25	0.019
	40	0.30	0.021	0.098	0.0064	0.26	0.020
	80	0.26	0.016	0.074	0.0049	0.18	0.015
	120	0.18	0.014	0.053	0.0039	0.13	0.011
	160	0.12	0.009	0.042	0.0031	0.11	0.0089
+ Thiamine	0	0.41**	0.032**	0.120**	0.0092**	0.40**	0.032**
(50 ppm)	40	0.42**	0.030**	0.125**	0.0090**	0.39**	0.031**
	80	0.36**	0.027**	0.110**	0.0075**	0.30**	0.026
	120	0.27**	0.022**	0.080**	0.0062**	0.29**	0.020
	160	0.20**	0.017**	0.061	0.0047*	0.19*	0.015
+ Ascorbic acid	0	0.45**	0.028*	0.135**	0.0099**	0.37**	0.030**
(50 ppm)	40	0.43**	0.031**	0.132**	0.0100**	0.35**	0.028**
	80	0.32	0.024**	0.103**	0.0070**	0.28**	0.021*
	120	0.25	0.020*	0.078*	0.0060**	0.20*	0.017*
	160	0.20	0.018**	0.069**	0.0050**	0.17	0.013
$LSD_{5\%}$		0.075	0.005	0.020	0.0014	0.065	0.0051
$LSD_{1\%}$		0.100	0.007	0.027	0.0019	0.088	0.0069

* Differences significant at the P = 0.05 level compared to the control
** Differences significant at the P = 0.01 level compared to the control

the three experimental species in comparison with that of the corresponding seedlings treated only with NaCl. Moreover, the vitamins completely counteracted the inhibitory effects of NaCl on fresh and dry matter production, especially at lower and moderate salinization levels. This counteraction was more pronounced in the case of *Lens culinaris* plants.

The data in Table 3 reveal that salinity induced an increase in the contents of soluble proteins in the case of *Cicer arietinum* and *Lens culinaris* plants. This increase reached its highest value at the level of 120 mM NaCl in *Cicer* seedlings, while in the case of *Lens* seedlings the highest value was recorded at the 160 mM NaCl level. On the other hand, *Trigonella* seedlings exhibited a decrease in the accumulation of soluble proteins with rising salinity levels. Vitamin treatments stimulated the accumulation of soluble proteins in the seedlings of the three test plants compared with those subjected only to NaCl. This accumulation was more pronounced in the case of thiamine than for ascorbic acid in all the test plants except for *Cicer arietinum* seedlings, where this effect was clearer after using ascorbic acid, especially at higher levels (120 and 160 mM) of NaCl.

Total free amino acids and proline were progressively accumulated in the three types of test seedlings with rising salinity levels (Table 3). In most cases, vitamin treatments also stimulated the accumulation of free amino acids as compared with the corresponding untreated plants, whatever the plant species tested. By contrast proline accumulation was obviously inhibited by vitamin treatments. This inhibition was more obvious in *Cicer* than in the other test plants and was also more obvious in the case of thiamine than for ascorbic acid.

Salinity induced a marked, progressive decrease in the contents of soluble carbohydrates in *Cicer* and *Lens* seedlings, except at the level of 40 mM NaCl, where a positive accumulation of soluble carbohydrates was exhibited (Table 3). On the other hand, salinity exhibited a reversible effect on the soluble carbohydrates accumulation in *Trigonella* seedlings, since the highest value was recorded at the level of 120 mM NaCl.

Vitamin treatments markedly stimulated the accumulation of soluble carbohydrates in all seedlings of the species as compared with that in untreated plants. On the other hand, in the case of *Lens culinaris* the effect of ascorbic acid was more obvious than that of thiamine, whereas in the case of *Trigonella* seedlings thiamine was more effective.

Discussion

Salinity induced a significant reduction in seed germination, radicle and plumule lengths, and fresh and dry matter production in *Cicer arietinum*, *Lens culinaris* and *Trigonella foenum-graecum* seedlings. These effects of salinity are in accordance with those obtained by Heikal and Shaddad (1982), Ahmed et al. (1980), Khan and Naqvi (1984), Shaddad et al. (1990), Lin and Kao (1996) and Azooz (1997). This inhibitory effect of salinity on seed germination and seedling growth may be attributed to the accumulation of toxic ions and/or reduced water uptake which arrests radicle emergence (Kurth et al., 1986; Hampson and Simpson,1990; Bengum et al., 1992).

Table 3

Interactive effects of salinity and seed soaking in thiamine or ascorbic acid on the production of soluble carbohydrates, soluble proteins, free amino acids and proline (mg g^{-1} dry matter) in *Cicer arietinum*, *Lens culinaris* and *Trigonella foenum-graecum* seedlings

Treatments	NaCl (mM)	*Cicer arietinum*				*Lens culinaris*				*Trigonella f. graecum*			
		Soluble sugars	Soluble proteins	Free amino acids	Proline	Soluble sugars	Soluble proteins	Free amino acids	Proline	Soluble sugars	Soluble proteins	Free amino acids	Proline
Reference control	0	41.10	65.10	28.40	3.00	26.17	55.50	43.81	9.00	22.10	80.30	40.77	4.35
	40	42.37	71.23	29.67	3.91	26.22	58.50	49.43	9.27	21.31	78.80	43.99	5.41
	80	38.31	78.41	33.22	4.69	20.25	62.40	58.29	10.49	23.10	73.92	53.99	5.47
	120	35.81	84.23	35.00	4.94	19.37	68.20	64.02	11.21	26.10	70.50	64.00	5.82
	160	32.91	71.90	30.10	5.93	15.30	72.00	67.15	12.34	24.29	70.30	64.49	5.60
+ Thiamine	0	47.17**	79.90**	33.52**	2.74	28.60*	62.32*	61.94**	9.93*	28.79**	91.30**	57.50**	4.12
(50 ppm)	40	48.10**	95.33**	36.87**	3.21*	29.36**	71.95**	62.10**	7.99**	23.18	87.00**	57.49**	4.67**
	80	46.61**	96.30**	39.20**	3.39**	25.35**	78.50**	62.96	9.67*	27.44**	84.80**	62.10**	4.73**
	120	43.95**	92.77**	43.50**	3.63**	24.00**	83.20**	71.79*	10.48	30.15**	80.68*	73.67**	4.93**
	160	40.92**	89.40**	40.73**	4.74**	22.34	88.35**	74.84**	10.82**	32.22**	79.00**	69.50	5.09**
+ Ascorbic acid	0	44.34**	76.70**	35.92**	2.53	32.18**	60.24	65.72**	10.50**	25.00**	87.95**	53.48**	4.23
(50 ppm)	40	44.62**	88.52**	35.29**	3.30	32.78**	68.35**	65.77**	8.76*	23.61*	84.33**	56.90**	4.86**
	80	42.84**	93.96**	43.18**	3.42**	28.23**	75.68**	69.91**	9.81	25.45*	82.00**	57.49	4.97**
	120	40.44**	96.25**	46.29**	3.77**	26.11**	80.10**	74.54**	10.82	28.95**	78.30**	72.34**	5.14**
	160	38.06**	92.91**	41.23**	4.92**	22.88**	85.20**	76.35**	10.87**	30.27**	75.67**	65.76	5.20**
$LSD_{5\%}$		2.23	5.01	2.65	0.63	2.27	6.24	5.00	0.79	1.91	3.34	6.09	0.31
$LSD_{1\%}$		2.99	6.75	3.57	0.85	3.73	8.41	6.73	1.06	2.57	4.50	8.19	0.42

* Differences significant at the P = 0.05 level compared to the control

** Differences significant at the P = 0.01 level compared to the control

The soaking of seeds presowing in thiamine or ascorbic acid alleviated the suppressive effects of the relatively higher salinity levels on the seed germination, radicle and plumule length, and fresh and dry matter yield of the three experimental plants. It appears probable from this response that the two vitamins used in this study may act as growth stimulants (Greulach and Adams, 1963) which can play a role in reversing the effect of NaCl on metabolic activities relevant to growth by enhancing cell division and/or cell enlargement. This enhanced cell division and cell enlargement would result in larger radicles and plumules, consequently increasing the fresh and dry yields of the seedlings, presumably as a result of the larger surface area available for anabolic activities. Such promoting effects of vitamins on the growth rate were also obtained by other authors (Khan and Ansari, 1984; Ansari and Khan, 1986; Shaddad et al., 1990; Mozafar and Oertli, 1992; Azooz, 1997).

Free amino acids, including proline, accumulated progressively in the three test seedlings as the salinity level was raised. These results are in agreement with those obtained by Stewart and Larher (1980), Ashraf and Fatima (1995) and Morabito et al. (1996). These results showed that the pronounced accumulation of proline was not at the expense of other free amino acids, which is in agreement with the results obtained by Widholm (1988) and Madan et al. (1995). Therefore, it may be concluded that the accumulation of proline and other free amino acids is one of the major physiological mechanisms of salt tolerance in these plants. Treatments with thiamine or ascorbic acid markedly lowered the proline content and strongly elevated the accumulation of other free amino acids in seedlings of all the plants tested. This is in accordance with the results obtained by other authors (Shaddad et al., 1990; Azooz, 1997).

With *Cicer* and *Lens*, salinity induced a progressive increase in the amounts of soluble proteins. In *Trigonella* seedlings, on the other hand, soluble carbohydrates increased with increasing salinity in the culture medium. This increase in soluble components may in turn play an important role in increasing the osmotic pressure of the cytoplasm, a conclusion which is in accordance with the results obtained by Flowers et al. (1977) on halophytic plants and Drossopoulos et al. (1987), Shaddad et al. (1990) and Ashraf and Fatima (1995) on glycophytic plants. On the other hand, Handa et al. (1983) found when using cultured tomato cells adapted to water stress that the concentration of reducing sugars in the cells increased with adaptation to salinity concentrations as high as 600 mM in the cells. In accordance with this it can be said that *Trigonella* is fairly well adapted to salinity.

In the present study, the soluble proteins and carbohydrates in the three species of test seedlings remained more or less unchanged at a low level of NaCl (40 mM). However, at higher salinity levels the losses in carbohydrates in *Cicer* and *Lens* seedlings were accompanied by an increase in soluble proteins, whereas in *Trigonella* an opposite effect occurred. This leads to the conclusion that salt tolerance may be linked with an equilibrium and interconversion between carbohydrates and the nitrogen metabolism. Also, this observation confirms the osmo-regulatory role of organic solutes in increasing the ability of

these plants to absorb water and consequently maintain a constant tissue water content. In addition, it can be said that the strategy of osmotic adjustment varied in the test seedlings. In *Cicer* and *Lens* the osmotic adjustment was mediated by soluble proteins, but in *Trigonella* by soluble carbohydrates.

The marked increases in soluble carbohydrates and soluble proteins in vitamin-treated plants may indicate that vitamins are able to alleviate the imposed salt stress via the stimulation of carbohydrate and protein synthesis. In accordance with this, Kodandaramaiah (1983) recorded significant alterations in the enzymes related to carbohydrate and protein metabolism, indicating that vitamins might act as activators of carbohydrate and protein synthesis. Gopala Rao and Sundrasanam (1984) observed an increase in α-amylase activity in green gram seedlings as a result of vitamin treatments. In addition, vitamin treatments increased the protein content in cluster bean plants (Gopala Rao et al., 1987). Moreover, vitamins might act at the translation level of protein synthesis (Gopala Rao et al., 1975; Arrigoni et al., 1977).

Generally, the results obtained in this study demonstrated that when these salt-stressed plants were treated with thiamine or ascorbic acid, the accumulation of carbohydrates and proteins was considerably raised, while contents of the amino acid proline were often reduced. This means that the incorporation of amino acids into protein was markedly enhanced when the test plants were treated with vitamins. This promoting effect on protein and carbohydrate contents was closely associated with a marked increase in seedling growth and dry matter yield. This would suggest that the depressive effects of salt stress on seed germination and seedling growth and other relevant physiological activities can be alleviated and/or modified to some extent by soaking the seeds presowing in appropriate concentrations of ascorbic acid or thiamine.

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EFFECT OF LARGE DOSES OF NITROGEN AND POTASSIUM FERTILISERS ON THE CRUDE PROTEIN CONTENT AND AMINO ACID COMPOSITION OF POTATO

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The potato varieties Russet Burbank, Desiree and Őszi rózsa were used in a two-year arable farm experiment to study the influence of variety characteristics, nutrient supplies and the growing season on the crude protein content, amino acid composition and essential amino acid index (EAAI) of potato.

It was found that with the fertiliser doses examined, the genotype had a more significant effect on the dry matter, N% (crude protein) and EAAI value than the application of mineral fertilisers; consequently, breeding might lead to better results than increased doses of fertilisers. Increasing doses of N were found to cause a rise in the amount of crude protein; however, an increase in crude protein led to a decrease in the EAAI, i.e. the quality of the crude protein deteriorated. In the varieties examined, methionine was identified as the limiting amino acid. The quantity of lysine decreased to the greatest extent as the N% rose. The application of N fertilisers caused the greatest increase in the amount of aspartic acid. Among the varieties examined, Desiree had the lowest EAAI value.

Key words: potato, nutrient supply, dry matter, crude protein, amino acid composition, essential amino acid index

Introduction

Different potato varieties have an average 1.5–2% crude protein content in their tubers. In comparison with other crops this is a relatively low value; however, if the crude protein production on an area of one hectare is calculated, taking world average yields into consideration, potato (273 kg protein/ha) takes second place, preceded only by peas (304 kg protein/ha) (Winiger, 1978). According to Niederhauser (1993), on world average, the protein production of potato per ha exceeds that of maize, wheat and rice. As regards quality potato protein is very valuable due to its higher methionine content, so it is of better quality than the protein of peas or maize. In comparison with cereals, it has a higher lysine content, and according to some researchers, it has a similar composition to animal proteins (Kaldy and Marakis, 1972; Rexen, 1976). There is thus a call to increase the available quantities of this biologically valuable protein. In the first step all the factors and correlations which might have an influence on the protein content and amino acid composition of potato should be pinpointed and investigated.

From the point of view of processing, the amino acid composition of potato is of significant importance. For example, to minimise the Maillard effect during frying, tyrosine and proline should be present in abundance, while only small quantities of leucine and isoleucine should be present (Holm, 1974).

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From among the agrotechnical factors, it is the nutrient supply, especially the quantity of nitrogen, that has the greatest influence on the protein content and the amino acid composition. However, it must not be forgotten that an increase in the amount of crude protein may result in a decrease in the biological value of the proteins (Kralovánszky, 1975). In this respect, a well-balanced nutrient supply as regards the proportion of nutrients (micro- and macroelements) may have a significant modifying effect (Müller, 1965; Németh and Keresztes, 1969).

Breeding for high protein content has been underway for a number of years. According to several authors, the transmittal of protein content is polygenic (Veilleux et al., 1981). There were attempts to raise the protein content of potato at the Department of Potato Research in Keszthely as early as the 1970s. With the use of crossbreeding and mass selection, utilising some wide genotypes, Sárvári et al. (1977) found several breeding lines possessing good agronomic qualities, with 4.5% protein content per raw tuber, which were also resistant to a number of pathogens; however, only 0.75% of all the materials examined contained more than 4% protein.

The development of breeding methods and the appearance of new biotechnological methods raise the possibility of modifying the protein content and amino acid composition more efficiently. The modification of the amino acid metabolism with the help of gene engineering, and the transformation of plants to give higher levels of essential amino acids or other valuable qualities are a realistic possibility these days (Tu et al., 1998; Chong et al., 1997; Utsumi et al., 1994).

Because of the above, protein and amino acid research in potato is still a considerable challenge. A two-year arable farm experiment was therefore set up to find an answer to the following questions: 1. What is the role of different N and K fertiliser doses, the growing season and the variety in the formation of dry matter, N% and the essential amino acid index (EAAI)? 2. Which are the limiting essential amino acids?

Materials and methods

The three-replication, small-plot experiments were carried out in Keszthely in 1994 and 1995, on the experimental field of the Department of Potato Research, on Ramann's brown forest soil. The experiments included the varieties Őszi rózsa, Desiree and Russet Burbank. The amounts of the different fertilisers applied are shown in Table 1.

At harvest, the yields were homogenised and 10 tubers were processed per variety and treatment. One quarter of the tubers was cut off lengthwise, grated and mixed, and the measured quantities were dried, re-measured (to determine the quantity of dry matter) and ground. The crude protein content and the amino acid composition were determined from this grist.

The Kjeldahl method was used to determine the N%, from which the quantity of crude protein was calculated by multiplying the result by 6.25.

After hydrolysing the samples, the amino acids were separated with ion-exchange column chromatography and detected with photometry. For the measurements a BIOTRONIK 5001 automatic amino acid analyser was used, with a nor-leucine inner standard.

Table 1
Fertiliser treatments applied in the experiments

Fertiliser	Treatments (kg/ha)							
	1	2	3	4	5	6	7	8
N	150	300	450	600	150	300	450	600
P_2O_5	150	150	150	150	150	150	150	150
K_2O	250	250	250	250	500	500	500	500
Total active agents	550	700	850	1000	800	950	1100	1250

The essential amino acid index (EAAI), was calculated with the method of McDonald et al. (1982). The index, which takes into consideration the quantities of nine essential amino acids (phenylalanine, leucine, isoleucine, threonine, methionine, lysine, valine, histidine and arginine), was defined as the geometric mean of the egg ratios of these acids, and was calculated as

$$\text{EAAI} = \sqrt[n]{\frac{a}{a_e} \times \frac{b}{b_e} \times \frac{c}{c_e} \times \text{K} \times \frac{i}{i_e}},$$

where a, b, c, ... i = concentrations of the essential amino acids in potato protein; a_e, b_e, c_e, ... i_e = concentrations of the same amino acids in egg protein, and n = the number of amino acids included in the calculation (McDonald et al., 1982).

The data were recorded and graphically depicted using the Excel 97 program and statistically processed with the SPSS 7.5 program package.

It should be mentioned that in this experiment the nutrient level was deliberately increased to three times the dose of N and twice the dose of K required for an average yield in order to strengthen the expected effects. When the samples from the arable farm experiments and later from storage experiments were processed, besides the crude protein and amino acid contents, measurements were also made on several other chemical characteristics, and on cooking technology and enzyme parameters; the K doses were applied mainly to allow the latter features to be studied. Because of lack of space, this paper is restricted to the evaluation of the dry matter, N% (crude protein) and amino acid data obtained from the autumn data processing.

Results

Some of the most important parameters measured and calculated in autumn are summarised in Table 2. The data prove that as regards dry matter content, the varieties showed a definite order; Őszi rózsa had the highest (23.62 – 25.11%) average dry matter content. One interesting result of the experiment was that, averaged over the treatments, the variety Desiree, which is outstanding from several points of view, had the lowest EAAI value of the three varieties, and its crude protein content was not outstanding either.

During the processing of the results, based on the data for the autumns of the two years, multivariable regression analysis was applied with the help of the SPSS 7.5 program. Regression methods were used to describe the relationship between dry matter, N% (crude protein) and the essential amino acid index as dependent variables, and the N, K fertiliser doses, varieties and growing season as independent variables. All three regression models were significant at the $P<0.001$ level. Based on the correlations the following statements can be made:

Table 2
Quantities of dry matter (%), crude protein (%) and EAAI in the different N/K treatments (see Table 1) in the autumn tests

Treatment	Dry matter (%)				*Crude protein (%)				EAAI			
	ŐR	D	RB	Average	ŐR	D	RB	Average	ŐR	D	RB	Average
						1994						
1	26.84	21.51	23.93	24.09	2.05	1.98	2.28	2.10	85.87	84.32	81.79	83.99
2	26.03	19.14	21.89	22.35	2.10	2.05	2.44	2.20	91.05	71.35	75.79	79.40
3	27.11	18.47	21.22	22.27	2.54	2.23	2.44	2.41	86.14	70.04	81.24	79.14
4	25.68	18.62	19.52	21.27	2.55	2.26	2.32	2.38	84.16	71.45	80.67	78.76
5	25.13	21.31	21.01	22.48	2.14	2.04	2.03	2.07	88.68	76.54	83.10	82.77
6	24.14	18.85	19.69	20.89	2.22	1.99	2.15	2.12	89.07	72.52	80.16	80.58
7	22.33	18.94	19.11	20.13	2.17	2.12	2.21	2.17	88.34	78.58	81.52	82.81
8	23.61	16.14	20.08	19.94	2.32	2.13	2.30	2.25	84.78	69.36	76.76	76.97
Average	25.11	19.12	20.81		2.26	2.10	2.27		87.26	74.27	80.13	
						1995						
1	25.43	18.72	24.18	22.77	2.15	2.43	2.61	2.40	93.83	69.75	82.19	81.92
2	23.58	19.30	20.60	21.16	2.17	2.28	2.73	2.39	81.90	71.32	74.33	75.85
3	22.79	18.47	20.84	20.70	2.26	2.45	2.77	2.50	77.20	66.95	74.01	72.72
4	25.82	17.23	19.69	20.92	2.35	2.54	2.82	2.57	85.66	65.01	71.27	73.98
5	23.91	18.09	19.32	20.44	1.95	2.33	2.43	2.24	82.80	77.06	69.94	76.60
6	23.44	16.37	18.95	19.58	2.19	2.52	2.46	2.39	90.42	66.09	72.18	76.23
7	22.25	17.95	19.33	19.84	2.16	2.49	2.73	2.46	76.51	64.69	76.67	72.62
8	21.72	15.45	18.79	18.65	2.20	2.45	3.05	2.57	78.23	68.75	70.42	72.46
Average	23.62	17.70	20.21		2.18	2.44	2.70		83.32	68.70	73.88	

*Proportion of raw mass; ŐR: Őszi rózsa, D: Desiree, RB: Russet Burbank

The growing season did not seem to have a significant effect on the amount of dry matter. The variety characteristics had an outstanding effect ($P<0.001$), followed by the effect of different doses of K and then that of N fertilisers. The application of increased doses of both N and K decreased the quantity (%) of dry matter. The decrease in dry matter content resulting from K and N overdoses is a well-known phenomenon (Horváth, 1978). It was possible to explain 48.1% of the deviation in dry matter content by the change in the four independent variables examined ($R^2 = 0.481$).

The quantity of N (and thus that of crude protein) was significantly influenced by the other factors, except by the amount of K. Based on standardised, partial regression coefficients this influence was greatest for genotype, followed by growing season and N dose. Rising doses of both nutrients increased the quantity of N, but only the effect of N fertiliser was significant ($P<0.001$). The increase in the N% value in the dry matter of the different varieties as a function of the amount of fertiliser applied is shown in Figure 1. It can be seen that as a consequence of the increase in N doses, the N% of the dry matter increased. Here, too, the varieties exhibited a definite order, though at higher N doses (450–600 kg/ha) the N% of the variety Desiree occasionally exceeded that of Russet Burbank.

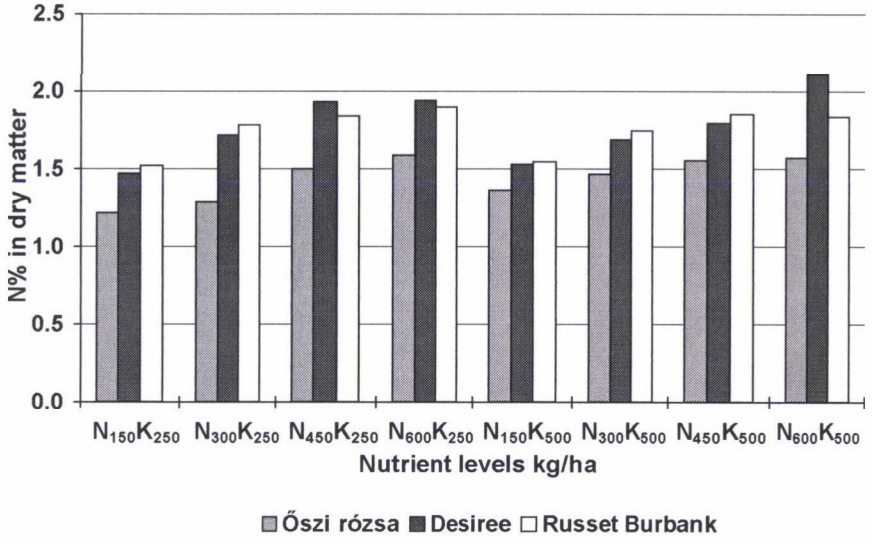

Fig. 1. Changes in N% as a function of nutrient levels (autumn 1994)

In the formation of the EAAI it was the genotype ($P<0.001$), the growing season and the N dose ($P<0.05$) that had a significant effect. Increasing N doses brought about a decrease in the EAAI value (Table 3).

The analysis of the data proved indubitably that increasing N doses brought about an increase in the quantity of crude protein; however, parallel with the increase in crude protein the essential amino acid index decreased, i.e. the quality deteriorated (Fig. 2). This statement is confirmed by the data in Table 4.

Table 3
Summary of regression correlations

n = 48		Growing season[a] (x_1)	Genotype[b] (x_2)	N, kg/ha (x_3)	K_2O, kg/ha (x_4)	For the whole model	
Y	a	b_1	b_2	b_3	b_4	R^2	P<
Dry matter %	31.003	–1.170	–1.928***	–0.0047*	–0.0068*	0.481	0.001
Regr. coeff. st.[c]		–0.199	–0.536	–0.267	–0.289		
N %	0.442	0.293***	0.245***	0.0008309***	0.0003246	0.695	0.001
Regr. coeff. st.[c]		0.425	0.581	0.405	0.118		
EAAI	99.612	–5.254**	–4.144***	–0.012*	–0.0024	0.409	0.001
Regr. coeff. st.[c]		–0.352	–0.454	–0.278	–0.04		

[a] year 1994 = 1, 1995 = 2, *** $P<0.001$,** $P<0.01$,* $P<0.05$
[b] Őszi rózsa = 1, Desiree = 2, Russet Burbank = 3
[c] Standardised partial regression coefficients, $y = a + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4$

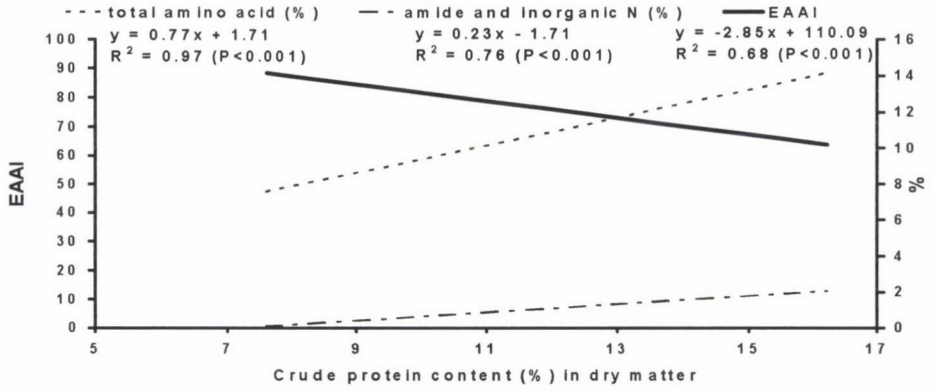

Fig. 2. Essential amino acid index (EAAI), total amino acids (%), amide and inorganic N fraction (%) as a ratio of crude protein (3 varieties, autumns of 2 years, n=48)

Table 4a
Total essential amino acids at harvest as a percentage of dry matter

Treatment	Őszi rózsa		Desiree		Russet Burbank	
	1994	1995	1994	1995	1994	1995
$N_{150}K_{250}$	3.61	4.26	4.25	5.13	4.38	4.97
$N_{300}K_{250}$	3.94	4.18	4.35	4.68	4.73	5.53
$N_{450}K_{250}$	4.41	4.25	4.77	4.98	5.15	5.51
$N_{600}K_{250}$	**4.55**	**4.27**	**4.86**	**5.41**	**5.28**	**5.78**
$N_{150}K_{500}$	4.05	3.78	4.12	5.50	4.46	5.03
$N_{300}K_{500}$	4.40	**4.56**	4.27	5.71	4.81	5.15
$N_{450}K_{500}$	**4.64**	4.17	4.94	4.93	**5.21**	5.97
$N_{600}K_{500}$	4.47	4.38	**5.17**	**6.08**	4.90	**6.32**

Table 4b
Total essential amino acids at harvest as a percentage of 16g of N

Treatment	Őszi rózsa		Desiree		Russet Burbank	
	1994	1995	1994	1995	1994	1995
$N_{150}K_{250}$	47.25	**50.38**	**46.14**	39.44	**46.05**	**46.02**
$N_{300}K_{250}$	**48.77**	45.40	40.52	**39.65**	42.38	41.65
$N_{450}K_{250}$	47.06	42.77	39.45	37.48	44.71	41.50
$N_{600}K_{250}$	45.78	46.86	40.09	36.67	44.49	40.36
$N_{150}K_{500}$	47.53	46.23	43.14	**42.76**	**46.09**	40.00
$N_{300}K_{500}$	**47.95**	**48.86**	40.52	37.03	44.08	39.68
$N_{450}K_{500}$	47.84	43.04	**44.02**	35.49	45.00	**42.22**
$N_{600}K_{500}$	45.51	43.23	39.25	38.36	42.72	38.97

Bold type is used to indicate the maximum values at different K levels

The quantity of essential amino acids accumulated in the dry matter at a K dose of 250 kg/ha showed a maximum value at the highest N dose (600 kg/ha). At a K dose of 500 kg/ha the maximum essential amino acid quantity was observed mainly at higher (450–600 kg/ha) doses of N (Table 4a). The N fertiliser increased the absolute quantity of essential amino acids in the dry matter. However, as shown in Table 4b, within the crude protein the quantity of essential amino acid reached a maximum at smaller (150–300 kg/ha) doses of N; i.e. large doses of N (450–600 kg/ha) decreased the proportion of essential amino acids in the crude protein.

When analysing the amino acid composition of the different samples, methionine was identified as the limiting amino acid in all three varieties, regardless of the nutrient supply. The next two limiting amino acids were isoleucine and leucine.

If the amount of the different amino acids is examined in correlation with the N%, it can be stated that for all three varieties it was the quantity of lysine that showed the greatest decrease with an increase in N% (Fig. 3 and Table 5). The determining coefficient value (R^2) of the linear regression equations describing the decrease in lysine is 0.44–0.79, which shows a medium or strong negative correlation between lysine and the N%. The next greatest decrease was found for different amino acids in the different varieties: for Őszi rózsa the order was lysine, phenylalanine, arginine, and for Desiree and Russet Burbank, lysine, histidine, leucine.

Only a few of the 17 amino acids examined showed an increase with an increase in N%. Among these only aspartic acid showed an outstanding, statistically significant increase. The data in Table 5 also confirm that the amount of methionine, which is considered to be a limiting amino acid, cannot be increased by increasing the N%, caused by N fertilisation.

The findings of the detailed amino acid tests confirmed the results reported by Rexen (1976) with other varieties, showing that the limiting amino acids of potato are methionine, lysine and isoleucine, and that as a result of increased N fertilisation, the quality of the crude protein deteriorates, especially due to the decrease in lysine. At the same time, in contrast with Rexen's findings, the present data showed that the glutamic acid accumulation brought about by N fertilisation was not of general validity; this was hardly experienced for Őszi rózsa.

Conclusions

The multivariable regression models confirmed that – at the fertiliser doses examined – the genotype had a more significant effect on the formation of dry matter, N% (crude protein) and EAAI value than fertilisation. This suggests that in the case of potato, breeding should be given greater emphasis in order to increase the crude protein content. Large doses of both K and N decreased the amount of dry matter. Increasing doses brought about an increase in the quantity of crude protein and essential amino acids within the dry matter, but at the same

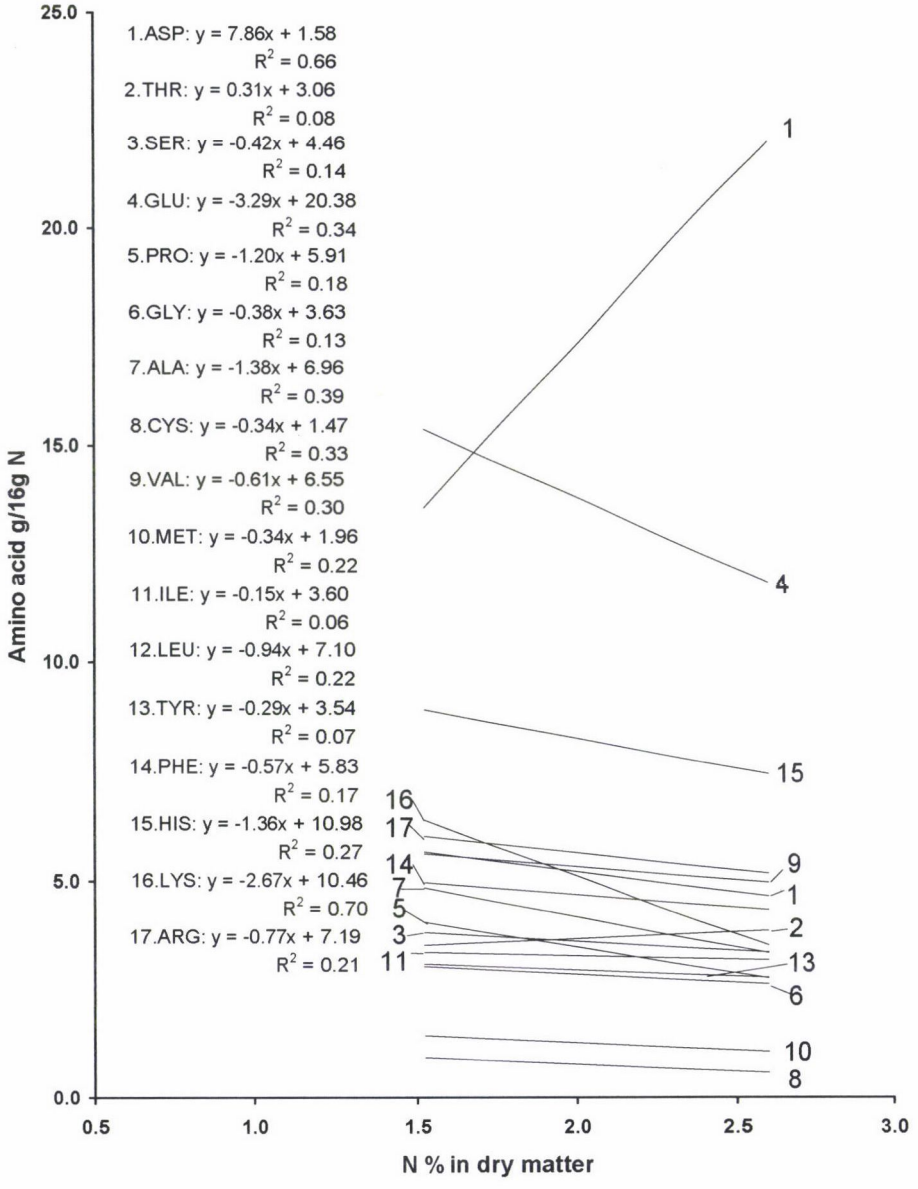

Fig. 3. Changes in the quantity of amino acids in the variety Russet Burbank as a function of N% (based on the harvesting data of 2 years, n=16)

Table 5
Regression equations describing changes in amino acid quantities as a function of N%

Amino acid	Őszi rózsa	Desiree	Russet Burbank
Aspartic acid	$y = 9.14x + 1.15$ $R^2 = 0.61$	$y = 5.73x + 7.53$ $R^2 = 0.37$	$y = 7.86x + 1.58$ $R^2 = 0.66$
Threonine*	$y = -1.80x + 6.73$ $R^2 = 0.19$	$y = -0.37x + 3.92$ $R^2 = 0.14$	$y = 0.31x + 3.06$ $R^2 = 0.08$
Serine	$y = -1.84x + 6.69$ $R^2 = 0.14$	$y = -0.26x + 3.76$ $R^2 = 0.04$	$y = -0.42x + 4.46$ $R^2 = 0.14$
Glutamic acid	$y = 3.58x + 7.53$ $R^2 = 0.14$	$y = -4.84x + 24.13$ $R^2 = 0.47$	$y = -3.29x + 20.38$ $R^2 = 0.34$
Proline	$y = -2.69x + 8.48$ $R^2 = 0.08$	$y = -2.29x + 8.28$ $R^2 = 0.51$	$y = -1.20x + 5.91$ $R^2 = 0.18$
Glycine	$y = -1.09x + 5.08$ $R^2 = 0.24$	$y = -0.82x + 4.20$ $R^2 = 0.54$	$y = -0.38x + 3.63$ $R^2 = 0.13$
Alanine	$y = -1.64x + 6.84$ $R^2 = 0.17$	$y = -2.06x + 7.82$ $R^2 = 0.48$	$y = -1.38x + 6.96$ $R^2 = 0.39$
Cysteine	$y = -0.11x + 1.26$ $R^2 = 0.01$	$y = -0.01x + 0.89$ $R^2 = 0.00$	$y = -0.34x + 1.47$ $R^2 = 0.33$
Valine*	$y = -1.36x + 7.17$ $R^2 = 0.21$	$y = 0.19x + 4.60$ $R^2 = 0.01$	$y = -0.61x + 6.55$ $R^2 = 0.30$
Methionine*	$y = -0.47x + 2.33$ $R^2 = 0.05$	$y = -0.31x + 1.80$ $R^2 = 0.57$	$y = -0.34x + 1.96$ $R^2 = 0.22$
Isoleucine*	$y = -0.53x + 4.45$ $R^2 = 0.04$	$y = -0.44x + 4.16$ $R^2 = 0.18$	$y = -0.15x + 3.60$ $R^2 = 0.06$
Leucine*	$y = 1.51x + 4.08$ $R^2 = 0.08$	$y = -1.24x + 7.01$ $R^2 = 0.33$	$y = -0.94x + 7.10$ $R^2 = 0.22$
Tyrosine	$y = -0.84x + 4.35$ $R^2 = 0.04$	$y = -0.17x + 3.57$ $R^2 = 0.03$	$y = -0.29x + 3.54$ $R^2 = 0.07$
Phenylalanine*	$y = -2.46x + 8.88$ $R^2 = 0.30$	$y = -0.90x + 5.86$ $R^2 = 0.50$	$y = -0.57x + 5.83$ $R^2 = 0.17$
Histidine*	$y = -0.63x + 9.09$ $R^2 = 0.02$	$y = -1.58x + 11.07$ $R^2 = 0.32$	$y = -1.36x + 10.98$ $R^2 = 0.27$
Lysine*	$y = -2.79x + 10.18$ $R^2 = 0.44$	$y = -2.14x + 9.26$ $R^2 = 0.79$	$y = -2.67x + 10.46$ $R^2 = 0.70$
Arginine*	$y = -2.12x + 9.19$ $R^2 = 0.39$	$y = -0.42x + 6.71$ $R^2 = 0.09$	$y = -0.77x + 7.19$ $R^2 = 0.21$

(*essential amino acid)

time the biological value of the crude protein decreased, as shown by the decrease in the essential amino acid index, the EAAI. According to the detailed amino acid tests, the quantity of essential amino acids decreased or stagnated as a function of the N% increase in the tuber, with lysine showing the most pronounced decrease. For all three varieties methionine was identified as a limiting amino acid, the amount of which cannot be significantly influenced by mineral fertilisation. Thus, an increase in the methionine content of potato should be achieved with other, preferably biotechnological methods. Increased doses of N fertilisation led to an increase mainly in the amount of aspartic acid. The fact that in this experiment the lowest EAAI values were found for the variety Desiree, which is otherwise known to have outstanding qualities, calls for a revision of views.

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EXAMINING PENETRATION RESISTANCE ON BROWN FOREST SOIL IN GÖDÖLLŐ

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In a long-term field experiment on soil management, started in 1994, the change in soil penetration resistance was examined during the vegetation period of 1997. The soil of the location was a brown forest soil of medium yield potential with a shallow fertile layer and moderate supplies of nutrients. In the present study the effect of five soil management variants on penetration resistance was evaluated.

According to the analysis of variance among the treatments it can be concluded that differences arising from the effect of cultivation can be experienced only in the 0–40 cm soil layer. The application of direct sowing and disking over several years has resulted in significantly higher penetration resistance values compared to those recorded after procedures combined with ploughing and loosening.

The resistance value of the compacted soil layer measured in early spring had an influence on the values measured later. In treatments involving disking, a penetration resistance of 4.7 MPa, found in spring under the cultivated layer, increased to 8.4 MPa by August. This increase was 1.5 MPa in treatments involving ploughing combined with loosening.

The results suggest that disking degrades the soil structure to the greatest extent and that the soil is least able to resist the degradation processes caused by disking.

Key words: soil tillage, penetration resistance, soil compaction

Introduction

The basic purpose of the soil is to supply crops with air and nutrients. It is only able to fulfil this function entirely if the proportions of the three phases (solid, gaseous, liquid) are satisfactory. If the volume weight of the soil exceeds 1.5 $g \cdot cm^{-3}$ and its penetration resistance reaches 2.5–3 MPa, it is qualified as destructively compacted soil (Eitzinger, 1991; van Ouwerkerk and Soane, 1994; Birkás, 1995). Hungarian (Stefanovits, 1994; Birkás et al., 1996; Várallyay, 1996) and foreign (Taylor, 1987; Beese, 1990; Sommer, 1990; Oldeman, 1994; Dannowski, 1995) authors agree that the physical degradation of the soil, including soil compaction, has the greatest tendency to cause damage and is the most difficult to prevent of all the degradation processes threatening the soil. Apart from natural factors (weather, sedimentation) it may be the consequence of disking or ploughing performed at the same depth for several years or of cultivating wet soil (Birkás, 1995).

The authors have been carrying out surveys on soil conditions in different agricultural regions of Hungary since 1987. The results show that compacted soil layers can be found at a certain depth or at several depths in the soils of 73%

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of this area, preventing undisturbed plant development and the infiltration of precipitation. Várallyay et al. (1989) classed Hungarian soils in 8 categories according to the susceptibility of the soil to physical degradation. Besides compaction, great emphasis was laid on structural stability and on resistance to wind and water erosion.

The value of penetration resistance as a key index expressing the relative extent of compaction depends on several factors. In addition to soil cultivation machinery and the measuring instruments applied, several physical and chemical soil parameters (volume weight, pore size distribution, soil particle-size distribution, organic matter content, soil water content, etc.) also influence the penetration resistance of the soil (Knittel and Stanzel, 1976; Schrey, 1991; Liebhard et al., 1995). According to Gardner (1972) the water content of the soil has the greatest influence on penetration resistance.

Both theoretical and practical soil science experts have drawn attention to the negative consequences of destructive soil compaction. Stefanovits (1992) pointed out disorders in the water, air and temperature cycles, while Gyuricza and Sipos (1996) indicated disadvantageous changes in microbiological activity and an increase in humus decomposition. According to Madas (1985) the decomposition of crop residues, manure and nutrients has slowed down, as has the nutrient and water absorption of the plants. Jóri (1990) demonstrated an increase in the energy needs of soil cultivation, while several authors (Raghavan, 1990; Kahnt, 1995) have noted the spread of diseases and pests, as well as a decrease in the resistance of the soil to erosion and deflation.

Hungarian and foreign authors have primarily evaluated the disadvantageous effects of soil cultivation – performed at the same soil depth for several years – on soil conditions. Long-term experiments carried out by Liebhard et al. (1995) in Upper Austria proved the necessity of changing the ploughing and loosening systems yearly. The highest penetration resistance values following 4-year cultivation with disking and direct sowing were experienced by Fenyves (1997) on Ramann's brown forest soil, but the results of Köller (1995), who proved that compaction lessened in direct ratio to the decrease in soil cultivation, are in contradiction with this.

The present research had the following aims:

- To evaluate soil cultivation and nutrient supplies, as well as the grain yield and quality of maize and wheat,
- To examine the relationship between cultivation performed at the same depth for several years and the physical, biological and chemical conditions of the soil.

Some of the results have already been published (Fenyves, 1997; Birkás et al., 1997; 1998). This paper presents the penetration resistance results measured in 1997.

Materials and methods

At the Experimental Station of the Institute for Crop Production, Gödöllő University of Agricultural Sciences, changes in soil conditions were evaluated in a long-term trial started in 1994, in which maize was grown in rotation with winter wheat.

An early maize hybrid (FURIO SC) was grown in the experimental year. Two factors (soil cultivation and fertiliser use) were examined in a strip arrangement in three replications. The soil cultivation block in each replication was 105 m^2, while that of the fertiliser treatment had a 35 m^2 net area.

The following treatments were applied:

Soil management:
- DD = Direct drilling
- D = Disking (16–20 cm)
- P = Ploughing (22–25 cm)
- LD = Loosening (35–40 cm) + Disking (16–20 cm)
- LP = Loosening (35–40 cm) + Ploughing (22–25 cm)

Fertiliser use:
- F_1 = untreated (control)
- F_2 = 80 kg N + 45 kg P_2O_5 + 75 kg K_2O/ha
- F_3 = 160 kg N + 90 kg P_2O_5 + 150 kg K_2O/ha

The nitrogen was applied in the form of ammonium nitrate, the phosphorus as dicalcium phosphate, and the potash as oxide. As regards the nutrient supplies of the soil the F_2 treatment represented a low, and the F_3 an adequate fertiliser level. For each soil cultivation procedure measurements on penetration resistance were performed in the latter treatment.

Soil characteristics: the experimental area in Gödöllő is in the intersection of latitude 47° 46' north and longitude 19° 21' east, in the microregion of the Gödöllő Hills. The soil is Ramann's forest soil, the parent material of which is tertiary sand and marl, covered by loess to different depths. The soil texture is sandy loam. The clay content of the topsoil (0–40 cm) is 16.5%, with good hydraulic conductivity, while that of the subsoil is poor. The humus content of the topsoil is 1.0–2.5% depending on the soil cultivation and the nutrient supply, as well as on its nitrogen content. The total N is 0.14–0.17%, the Al-P_2O_5 is 120–283 mg/kg, while the Al-K_2O is 110–309 mg/kg. The pH_{KCl} value is 6.6–7.1; the Arany sticky point (K_A) is 27.

Weather conditions: average precipitation is 564 mm, of which 313 mm falls in the vegetation period. In this respect 1997 was typical, with a total of 303 mm precipitation during the vegetation period. The deviation from the average over many years was significant in the months of July (+46.8 mm) and October (–41.5 mm). Since the penetration resistance depends significantly on the moisture content of the soil, a study of the quantity of precipitation and its distribution over time is essential for the correct evaluation of the results.

The soil was examined with an electronic penetrometer of the Szarvas type during the vegetation period (Kocsis and Daróczi, 1995). The instrument is non-automatic, and is able to register the mechanical penetration resistance and the moisture content of the surface soil to a depth of 70–75 cm. During the measurements the probe, which terminates in a steel cone, is pushed into the soil by a tooth-pinion pressure structure. The probe transmits an electric signal proportionate to the pressure and to the moisture content during penetration into the soil as a function of depth. The instrument can store strength, depth and moisture content values per second.

A moving average was calculated to reduce the deviation. Repeated polynomial regressive analysis was carried out to interpolate curves to the depth of cultivation and to the maximum value of penetration resistance. To verify the effect of cultivation statistically, factorial analysis of variance was applied every 5 cm (Baráth et al., 1996).

Results and discussion

In the case of maize favourable soil conditions are characterised by values of 1.1–1.35 $g \cdot cm^{-3}$ volume weight and 1.2–3.0 MPa penetration resistance in the upper soil layer (0–40 cm) in brown forest soil.

Figures 1a–1c show the penetration resistance values to a soil depth of 60 cm, using the norms accepted in the literature, with the penetration resistance on the horizontal axis in MPa, and the soil depth on the vertical axis. The values of the moving averages are presented for each treatment and curves were fitted using multiple polynomial regressive analysis. The "y" values represent the penetration resistance, and the "x" values the soil depth raised to various powers (Tables 1a, 1b, 1c).

Figure 1a shows the penetration resistance of the soil cultivation treatments measured at the beginning of the vegetation period. Each measurement was performed in the 4th year of the trial.

After direct drilling without soil cultivation, linearly increasing resistance was experienced to a soil depth of 25 cm, with a maximum value of 4.57 MPa between 20 and 25 cm. The maximum of the curve was found 3–4 cm below this depth. In the 30–40 cm soil layer the penetration resistance values decreased by 1.5 MPa, while constant values were recorded below this depth. The tendencies were similar in the middle and at the end of the vegetation period. A well-marked change occurred in the 20–30 cm soil layer, where the penetration resistance values experienced at the beginning of the vegetation period increased by 25–30% as a function of time to 4.9 MPa in June and 6.28 MPa in August (relevant parts of Figs 1b and 1c). The thickening of the compacted layer was less pronounced than the horizontal increase in penetration resistance.

The measurements proved that disking had a significant compacting effect. Resulting from the shallow depth of cultivation, a penetration resistance greater than 3 MPa was measured even at a depth of 5 cm at the beginning of the vegetation period, which hindered sowing and the rapid germination of the maize. In the 20–25 cm soil layer this value exceeded 5 MPa, but in the whole of the surface soil (to a depth of 40–45 cm) it was never less than 3 MPa. By the middle and end of summer a considerable extension of the disk-pan could be experienced both horizontally and vertically. The 8.47 MPa resistance measured at the end of August not only forms an impenetrable layer for plant roots, but also brings about a drastic decline in biological activity. The soil resistance values in the upper 15–20 cm were significantly different from those in the direct sowing treatment. Below this depth differences owing to the effect of the treatment could not be revealed (Tables 2–4). By the end of the summer this was toue from a depth of 25–30 cm in consequence of the greater compaction caused by disking.

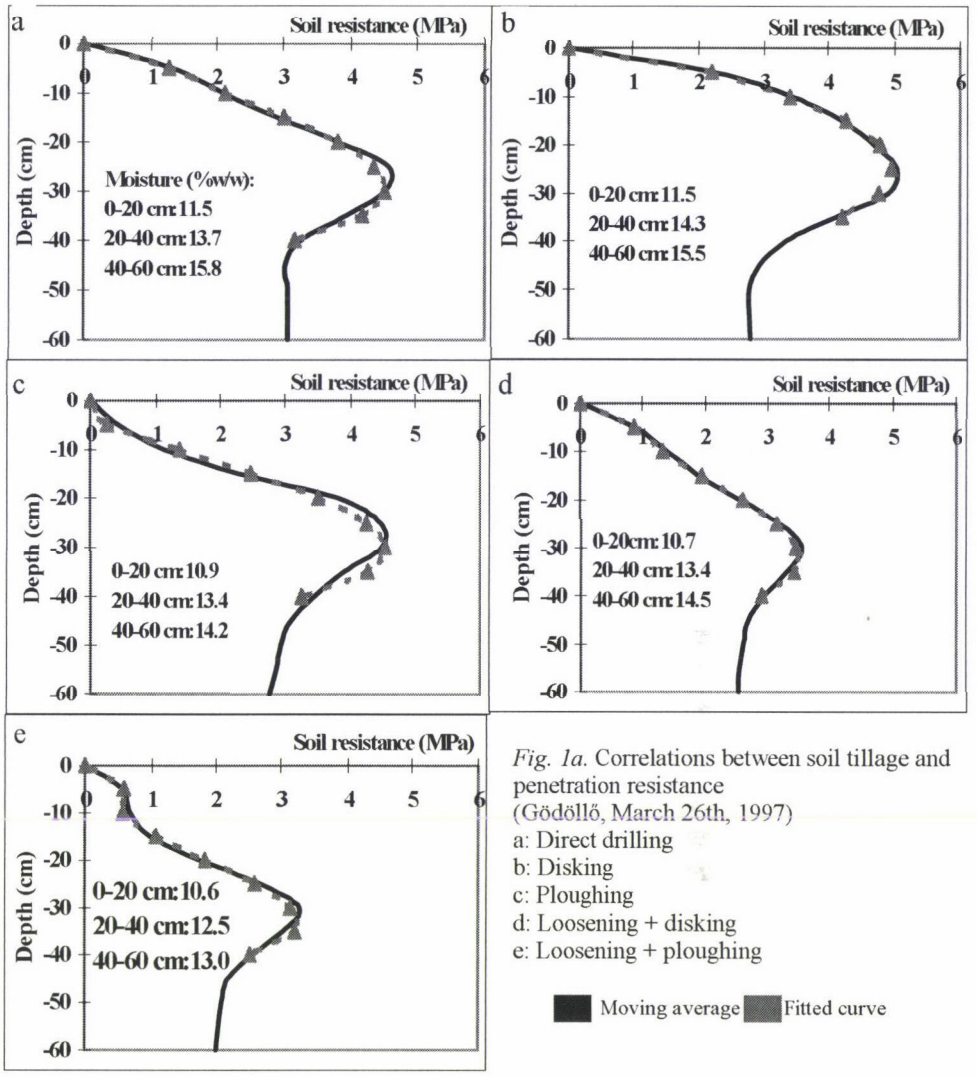

Fig. 1a. Correlations between soil tillage and penetration resistance
(Gödöllő, March 26th, 1997)
a: Direct drilling
b: Disking
c: Ploughing
d: Loosening + disking
e: Loosening + ploughing

Table 1a
Equations of the soil resistance curves fitted by multiple polynomial regression analysis in the soil tillage treatments* (March 26th 1997)

Soil tillage treatments	Equations
Direct drilling	$y = 0.566+0.114x+0.006x^2-0.0002x^3$
Disking	$y = 0.617+0.35x-0.0071x^2$
Ploughing	$y = 0.676+0.158x+0.0066x^2-0.0002x^3$
Loosening + disking	$y = 0.919-0.0534x+0.0114x^2-0.0002x^3$
Loosening + ploughing	$y = 1.37-0.25x+0.0203x^2-0.0004x^3$

*in the equations "y" represents soil resistance (MPa) and "x" soil depth (cm)

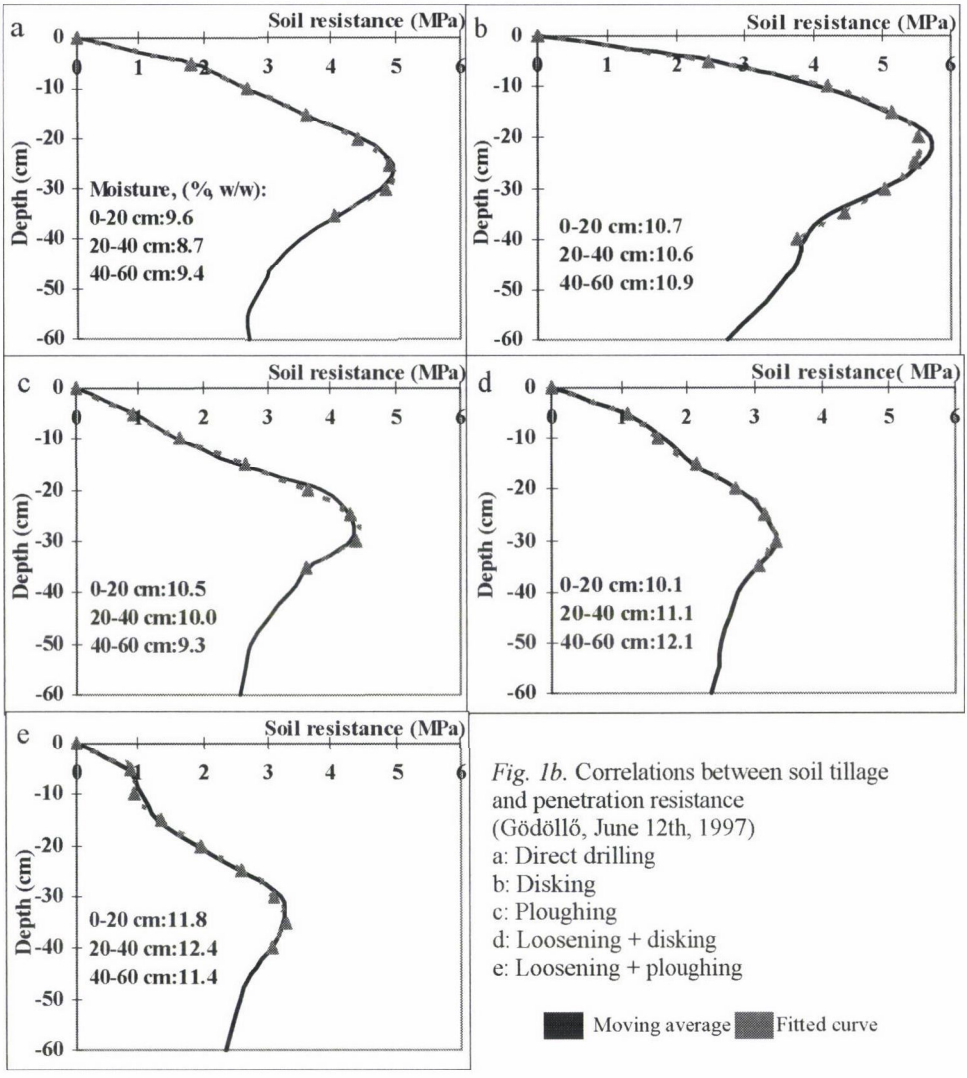

Fig. 1b. Correlations between soil tillage and penetration resistance
(Gödöllő, June 12th, 1997)
a: Direct drilling
b: Disking
c: Ploughing
d: Loosening + disking
e: Loosening + ploughing

Table 1b
Equations of the soil resistance curves fitted by multiple polynomial regression analysis in the soil tillage treatments* (June 12th 1997)

Soil tillage treatments	Equations
Direct drilling	$y = 1.37–0.25x+0.0203x^2–0.0003x^3$
Disking	$y = 0.043+0.6x–0.0194x^2+0.0002x^3$
Ploughing	$y = 0.764–0.045x+0.017x^2–0.0004x^3$
Loosening + disking	$y = 0.874+0.0096x+0.0076x^2–0.0002x^3$
Loosening + ploughing	$y = 1.32–0.151x+0.0134x^2–0.0002x^3$

*in the equations "y" represents soil resistance (MPa) and "x" soil depth (cm)

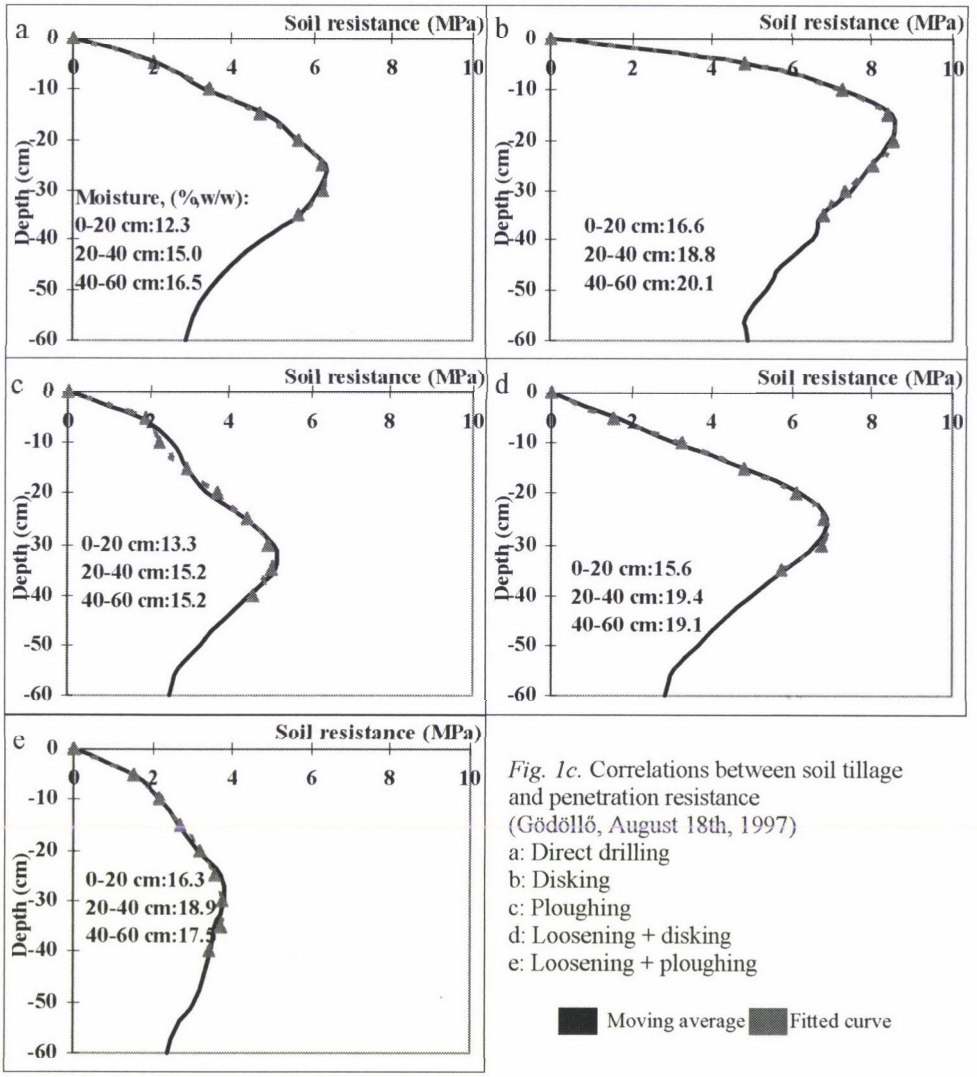

Fig. 1c. Correlations between soil tillage and penetration resistance
(Gödöllő, August 18th, 1997)
a: Direct drilling
b: Disking
c: Ploughing
d: Loosening + disking
e: Loosening + ploughing

Table 1c
Equations of the soil resistance curves fitted by multiple polynomial regression analysis in the soil tillage treatments* (August 18th 1997)

Soil tillage treatments	Equations
Direct drilling	$y = 0.497+0.296x+0.0011x^2-0.0002x^3$
Disking	$y= 0.643+1.03x-0.0418x^2+0.0005x^3$
Ploughing	$y = 1.98-0.071x+0.0124x^2-0.0002x^3$
Loosening + disking	$y = 0.056+0.29x+0.0068x^2-0.0003x^3$
Loosening + ploughing	$y = 0.761+0.136x+0.0005x^2-0.0001x^3$

*in the equations "y" represents soil resistance (MPa) and "x" soil depth (cm)

The values recorded after ploughing were in accordance with expectations. In the period preceding maize sowing, the penetration resistance measured in the upper 15 cm deep soil layer, which was 2.29 MPa at most, was favourable for the location in question. The compacted layer in the lower soil, below the depth of cultivation (4.48 MPa), was the consequence of ploughing at the same depth for several years. According to the early spring measurements the penetration resistance in the 20–30 cm soil layer after ploughing was lower, though not significantly, than after disking. The difference was significant in the upper soil layers (Tables 2–3).

According to examinations in June and August, the gradual increase in penetration resistance observed in the uppermost soil layer did not occur in the deeper soil layers (below 15 cm). While this increase was 2.1–4.3 MPa after disking and direct sowing, it was only 0–0.9 MPa after ploughing (Figs 1b–1c, Table 2). Consequently, disking had resulted in significantly higher penetration resistance values at all levels of the upper soil (0–40 cm) by the end of the vegetation period. The interaction between direct sowing and ploughing was also significant (except for the upper 10 cm soil layer). Below a depth of 40 cm there was no significant difference, as cultivation only had a direct influence in the upper layers.

The long-term application of ploughing and disking is essential not only for maize cultivation, but also for the cultivation of crops with a short root system (e.g. wheat) in traditional management systems. Several authors have detailed the effect of these techniques on the soil conditions (Liebhard et al., 1995; Fenyves, 1997; Birkás et al., 1998). Their conclusions correspond with the present results.

Procedures combined with loosening can be considered as soil-conserving methods owing to their ameliorative effect on soil conditions. Disking complemented with loosening to a depth of 35–40 cm improves material migration between the upper and lower soil layers, facilitates gas exchange and undisturbed infiltration and provides favourable conditions for the development of plant roots.

The results indicate that loosening has a beneficial effect. However, a compacted layer was formed below 20 cm (3.1–3.5 MPa) as a consequence of 4 years of shallow basic cultivation involving disking. In the seed-bed layer, resistance similar to that observed after ploughing was recorded. Later in the vegetation period a substantial increase in resistance was found to a depth of 45 cm. The highest value of penetration resistance exceeded 6.5 MPa at a depth of 28 cm. While significantly lower resistance could be recorded after disking combined with loosening in all the soil layers examined in March compared to direct sowing, disking and ploughing, the situation had changed to a certain extent by the end of the vegetation period: significantly lower compaction was measured after ploughing in the 10–25 cm soil layer. In the disking treatment higher resistance values were measured in all the layers examined, but significant changes were only found in the upper 20 cm (Tables 2–4).

Table 2
Trends in soil resistance and $LSD_{5\%}$ values at the beginning and end of the vegetation period at a depth of 0–60 cm (Gödöllő, 1997)

Depth (cm)	Beginning of the vegetation period (March 26th 1997)						End of the vegetation period (August 18th 1997)					
	DD	D	P	LD	LP	**$LSD_{5\%}$**	DD	D	P	LD	LP	**$LSD_{5\%}$**
0–5	1.30	2.21	0.41	0.87	0.55	**0.63**	2.02	4.80	1.77	1.60	1.45	**1.29**
5–10	2.11	3.45	1.12	1.40	0.67	**1.22**	3.31	7.27	2.57	3.05	2.13	**1.41**
10–15	2.89	4.22	2.29	1.89	0.97	**0.50**	4.76	**8.47**	2.97	4.88	2.66	**1.31**
15–20	3.80	**4.70**	3.79	**2.55**	1.74	**0.71**	5.58	**8.42**	3.46	**6.19**	3.17	**1.22**
20–25	**4.57**	**5.03**	**4.46**	3.19	**2.68**	**0.82**	**6.28**	7.98	**4.39**	**6.90**	**3.71**	**1.54**
25–30	4.50	4.90	**4.48**	**3.56**	**3.28**	**0.74**	6.13	7.48	5.07	6.59	**3.82**	**1.77**
30–35	3.93	4.13	3.90	3.31	3.03	**0.67**	5.67	6.75	**5.12**	5.83	3.58	**1.78**
35–40	3.26	3.35	3.45	**2.94**	**2.59**	**ns**	4.76	6.52	4.49	**5.02**	**3.49**	**1.70**
40–45	3.03	2.92	3.10	2.68	2.19	**ns**	3.98	5.72	3.89	4.29	3.30	**ns**
45–50	3.06	2.76	2.95	2.60	2.09	**ns**	3.44	5.37	3.34	3.64	3.06	**ns**
50–55	3.06	2.76	2.88	2.54	2.04	**ns**	3.05	4.84	2.74	3.07	2.60	**ns**
55–60	3.06	2.79	2.78	2.51	2.00	**ns**	2.83	4.90	2.52	2.83	2.38	**ns**

= Tillage depth = Maximum value

ns = Non-significant; DD = Direct drilling; D = Disking; P = Ploughing; LD = Loosening + disking; LP = Loosening + ploughing

Table 3
Soil resistance significance table between treatment pairs at the beginning of the vegetation period (Gödöllő, March 26th 1997)

	DD	**D**	**P**	**LD**		**DD**	**D**	**P**	**LD**
0–5 cm	$LSD_{5\%}$ = 0.63				**5–10 cm**	$LSD_{5\%}$ = 1.22			
D	0.91*				**D**	1.34*			
P	0.89*	1.8*			**P**	0.99	2.33*		
LD	0.43	1.34*	0.46		**LD**	0.71	2.05*	0.28	
LP	0.75*	1.66*	0.14	0.32	**LP**	1.44*	2.78*	0.45	0.73
10–15 cm	$LSD_{5\%}$ = 0.5				**15–20 cm**	$LSD_{5\%}$ = 0.71			
D	1.33*				**D**	0.9*			
P	0.6*	1.93*			**P**	0.1	0.91*		
LD	1.*	2.33*	0.4		**LD**	1.25*	2.15*	1.24*	
LP	1.92*	3.25*	1.32*	0.92*	**LP**	2.06*	2.96*	2.05*	0.81*
20–25 cm	$LSD_{5\%}$ = 0.82				**25–30 cm**	$LSD_{5\%}$ = 0.74			
D	0.46				**D**	0.4			
P	0.11	0.57			**P**	0.02	0.42		
LD	1.38*	1.84*	1.27*		**LD**	0.94*	1.34*	0.92*	
LP	1.89*	2.35*	1.78*	0.51	**LP**	1.22*	1.62*	1.2*	0.28
30–35 cm	$LSD_{5\%}$=0.67								
D	0.2								
P	0.03	0.23							
LD	0.62	0.82*	0.59						
LP	0.9*	1.1*	0.87*	0.28					

*=Significant; DD = Direct drilling; D = Disking; P = Ploughing; LD = Loosening + disking; LP = Loosening + ploughing

Table 4
Soil resistance significance table between treatment pairs at the end of the vegetation period (Gödöllő, August 18th 1997)

	DD	D	P	LD		DD	D	P	LD
0–5 cm	$LSD_{5\%}$ = 1.29				**5–10 cm**	$LSD_{5\%}$ = 1.41			
D	2.6*				**D**	3.96*			
P	0.25	3.03*			**P**	0.74	4.7*		
LD	0.42	3.2*	0.17		**LD**	0.26	4.22*	0.48	
LP	0.57	3.35*	0.32	0.15	**LP**	1.18	5.14*	0.44	0.92
10–15 cm	$LSD_{5\%}$ = 1.31				**15–20 cm**	$LSD_{5\%}$ = 1.22			
D	3.71*				**D**	2.84*			
P	1.79*	5.5*			**P**	2.12*	4.96*		
LD	0.12	3.59*	1.91*		**LD**	0.61	2.23*	2.73*	
LP	2.1*	5.81*	0.31	2.22*	**LP**	2.41*	5.25*	0.29	3.02*
20–25 cm	$LSD_{5\%}$ = 1.54				**25–30 cm**	$LSD_{5\%}$ = 1.77			
D	1.7*				**D**	1.35			
P	1.89*	3.59*			**P**	1.06	2.41*		
LD	0.62	1.08	2.51*		**LD**	0.46	0.89	1.52	
LP	2.57*	4.27*	0.68	3.19*	**LP**	2.31*	3.66*	1.25	2.77*
30–35 cm	$LSD_{5\%}$ = 1.78				**35–40 cm**	$LSD_{5\%}$ = 1.7			
D	1.08				**D**	1.76*			
P	0.55	1.63			**P**	0.27	2.03*		
LD	0.16	0.92	0.71		**LD**	0.26	1.5	0.53	
LP	2.09*	3.17*	1.54	2.25*	**LP**	1.27	3.03*	1	1.53

* = Significant; DD = Direct drilling; D = Disking; P = Ploughing; LD = Loosening + disking; LP = Loosening + ploughing

Ploughing combined with loosening resulted in the best soil conditions compared to the other treatments. The penetration resistance of 3.28 MPa measured at a soil depth of 25–30 cm at the beginning of the vegetation period did not increase to more than 3.8 MPa even in the second half of the vegetation period. Under the soil conditions of the experiment these values can be considered as acceptable. This was also obvious from the grain yield of maize: a surplus grain yield of almost 25% was harvested compared to direct sowing, which resulted in the lowest yield. After ploughing combined with 35–40 cm deep loosening a significantly lower penetration resistance was measured in the whole soil profile compared to direct sowing and disking. There was not significant difference between the two treatments involving loosening for the upper 10 cm layer, but below this level the difference was considerable. The increased compacting effect of disking was primarily observed in the 15–25 cm layer, which is the lowest depth of cultivation.

An examination of the effectiveness of soil loosening at the given location shows that though it lessens compaction temporarily, the use of machinery which leads to the development of cultivation-pan (disk, plough) over several vegetation periods significantly decreases its effect in the long term. This indicates the necessity of periodically changing the soil cultivation depth (Sipos, 1978; Liebhard et al., 1995).

According to the data in Table 2 the depth of cultivation and the position of the layers with the highest penetration resistance are clearly linked. Following disking and ploughing the most compacted layer is located, with only one exception, 0–10 cm below the typical cultivation depth. Techniques combined with loosening give similar results, except that the highest value is considerably lower than for cultivation without loosening.

The higher the penetration resistance of the soil layer at the beginning of the vegetation period, the greater the horizontal expansion of the compacted layer during summer. The penetration resistance of 5.0 MPa found below the cultivated layer at the end of March after disking had increased to 7.98 MPa by August. This increase was 1.5 MPa when disking was combined with loosening.

In the trial the value of penetration resistance was influenced primarily by the depth of cultivation and the cultivation equipment used.

The penetration resistance of the compacted layer increased in the following order after cultivation:

1. At the beginning of the vegetation period: Loosening+ploughing < Loosening+disking < Ploughing < Direct sowing < Disking

2. In the middle of the vegetation period: Loosening+ploughing < Loosening+disking < Ploughing < Direct sowing < Disking

3. At the end of the vegetation period: Loosening+ploughing < Ploughing < Direct sowing < Loosening+disking < Disking.

The penetration resistance values confirmed previous findings that disking degrades the soil structure to the greatest extent. The soil was least able to resist the degradation processes after disking.

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EFFECT OF CROPPING PATTERNS ON SOIL STRENGTH AND WATER CONTENT

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Soil strength and water content were measured in a long-term crop rotation experiment in Kompolt, Hungary. The main objective of the study was to examine different cropping systems and to compare their effect on mechanical impedance and water content with the use of penetration resistance. The results suggest that soil strength was greater in a long-term maize monoculture than in a crop rotation. Water content showed only slight differences between the two cropping patterns.

Key words: soil strength, cropping system, monoculture, crop rotation

Introduction

In crop production more and more emphasis is put on compaction problems. Soil compaction is considered to be a multidisciplinary problem in which machine–soil–crop–weather interactions play an important role (Soane and van Ouwerkerk, 1994). The soil tillage so important for arable farming is considered as a main factor in solving compaction, though it may often generate similar problems (Birkás et al., 1998; Jolánkai et al., 1997).

Soil tillage and plant nutrition are the two most important crop production factors (Nagy, 1993). Agricultural production is related to the environment. In sound systems cropping patterns have an important role (Ángyán and Menyhért, 1997). The level of farming has had a significant impact on soil conditions in Hungary in three major crops (Jolánkai et al., 1997; Birkás et al., 1995). When interpreting long-term cropping and tillage effects on soil conditions researchers often face several difficulties (Bartosová, 1998). Penetration resistance can be a good source of information not only in tillage experiments (Szalai et al., 1995; 1998). In long-term crop rotation experiments the importance of fertilization and the nutrient supply has been dominant (Berzsenyi and Győrffy, 1996; Holló, 1993; Sárvári, 1987).

The effect of the cropping system on soil strength or compaction depends on crop residues (Bullock, 1992); continuous maize resulted in higher bulk density (Hulugalle and Lal, 1986). Hageman and Shrader (1979) found the opposite tendency after 20 years of maize. In a long-term tillage – crop rotation experiment, after 10 years the crop rotation showed a non-significant influence on bulk density and soil impedance (Hammel, 1989).

The aim of the present study was to evaluate the level of soil strength under different cropping patterns. Penetration resistance and bulk density measurements are widely used in field trials having different tillage treatments. Less information is available from "pure" crop rotation studies.

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Materials and methods

Penetration resistance and water content (w/w) were measured using a penetrometer (Kocsis and Daróczi, 1995).

The long-term crop rotation experiment in which measurements were taken was set up in Kompolt in 1961 (Holló, 1993). The experiment has four replications and a split-split-plot randomized design. There are three cropping patterns on the main plots:

A. Maize monoculture

B. Maize–wheat double cropping (maize–maize–winter wheat–winter wheat)

C. Four–phase rotation (maize–spring barley-pea-winter wheat)

There are twelve levels of nutrient supply and on the sub-plots the after-effect of lucerne can also be studied. The size of each plot is 54 m^2. The soil type is chernozem brown forest soil, which is characteristic of more than 200,000 ha in the northern part of the Great Plain. The clay content is 46%; the depth of the water-table is 9–10 m.

In 1997 and 1998 five nutrient treatments were selected from the twelve. They represent the control, manure + fertiliser (34.5 t/ha FYM was applied in 1994 and every fourth year previously + 88, 44, 52 kg/ha N, P_2O_5 and K_2O, respectively), crop residue + fertiliser (maize and wheat straw was ploughed in after chopping + the same dose of fertilizers as above). Two other fertiliser treatments (1.5 times and twice the above dose) were also applied.

Soil strength and moisture content were measured in four replications in autumn.

This paper gives information on the 1997 results from main plots A and C.

Results and discussion

In the first year soil strength was measured on main plots A and C, since A was a 35-year continuous maize monoculture, while C represented a typical rotation.

Table 1 presents data on the soil strength (N/cm^2) at three depth categories: 11–30 cm, 31–50 cm and 51–80 cm. The top layer (0–10 cm) was not taken into consideration because agrotechnical activity and environmental effects may have influenced it. Soil tillage had direct effect to a depth of 0–25 cm.

Soil strength shows great variability at all depths, ranging from 233–509 N/cm^2 at 11–30 cm, 251–788 N/cm^2 at 31–50 cm and 262–623 N/cm^2 at 51–80 cm.

In the first depth category there was no consistent difference between the various nutrient treatments in the monoculture and the crop rotation. Significant differences occur, e.g. due to field differences, nutrient supply and rotation effects. However, the highest values (> 410 N/cm^2) were obtained on plots with a monoculture.

Below this layer annual tillage operations have only a minimal effect, so the impact of cropping patterns – if there are any and they can be measured – should be clearer. This layer shows higher compaction, with a maximum value of over 700 N/cm^2. It can be seen that the monoculture plots all had an impedance of 400 N/cm^2 or above with one exception, the control, which had only 360 N/cm^2 soil strength. In spite of the high LSD value, the data represent two main categories of soil strength, 251–379 N/cm^2 and 491–788 N/cm^2, corresponding to plots of the four-phase rotation and monoculture, respectively.

Table 1
Soil strength (N/cm^2) in a monoculture (A) and crop rotation (C) at three depths (Kompolt, 1997)

Depth 11–30 cm		Depth 31–50 cm		Depth 51–80 cm	
Plot	Mean	Plot	Mean	Plot	Mean
C/3 11	233.66	C/3 11	251.16	C/3 11	262.81
C/2 9	248.10	C/2 11	300.08	C/2 11	279.13
C/2 11	254.77	C/3 9	301.06	A/2 1	281.13
A/3 1	257.75	C/2 9	304.92	C/2 10	285.25
C/3 9	264.06	C/2 10	327.90	C/2 9	292.60
A/2 1	267.88	A/2 1	360.12	C/3 9	301.30
A/2 9	283.69	C/2 6	365.99	C/2 6	318.05
C/3 6	285.31	C/3 10	379.16	C/3 6	345.60
C/2 10	299.03	C/3 6	379.74	A/3 1	345.82
C/2 6	353.02	A/3 1	396.76	C/3 10	349.63
A/3 9	374.03	A/2 9	491.73	A/3 6	427.27
A/3 6	379.04	A/3 6	497.66	A/2 9	428.29
A/2 6	395.89	A/2 6	575.21	A/2 11	459.34
C/3 10	406.69	A/3 10	579.85	A/2 6	500.01
A/2 11	424.42	A/2 11	583.95	A/3 10	526.01
A/3 11	467.35	A/3 9	688.32	A/3 9	569.89
A/3 10	487.90	A/2 10	694.22	A/2 10	585.90
A/2 10	509.26	A/3 11	788.96	A/3 11	623.69
$LSD_{5\%}$	124.22	$LSD_{5\%}$	145.54	$LSD_{5\%}$	120.20

In the deepest layer (51–80 cm) this tendency did not change. The continuous maize plots exhibited higher values (427–623 N/cm^2), while the penetration resistance under different nutrient treatments of the crop rotation varied between 262–349 N/cm^2.

Figure 1 shows the tendency of soil strength at various depths, averaged over the cropping pattern.

The mean values represent a constant level of soil strength in the rotation. The differences are visible in the untilled layers.

The pre-crop and rotation effects are often dealt with from the point of view of water balance (Ruzsányi, 1991; Nyíri, 1993). Besides soil penetration resistance, information on the water content (w/w) is important, since it may influence impedance, too. On the other hand, if crop sequences have some advantage in water content the aim was to check if there were any differences under autumn conditions.

Table 2 presents data on the water content of the plots in the same depth categories as discussed in the case of soil strength.

In the deepest soil layer (51–80 cm) there was no significant difference between the plots. The water content varied between 29.02–30.4% (w/w). This meant that at maize harvesting no difference could be observed at this depth, while penetration resistance was not influenced by moisture content.

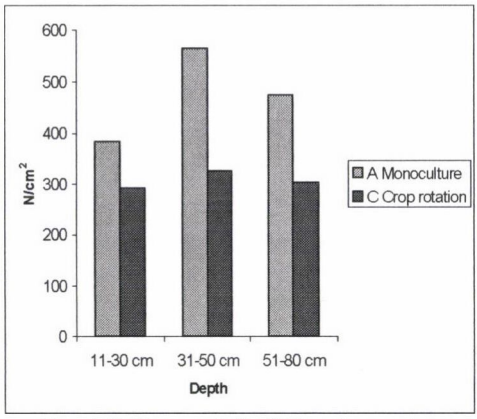

Fig. 1. Soil strength in different cropping systems at three depths (Kompolt, 1997)

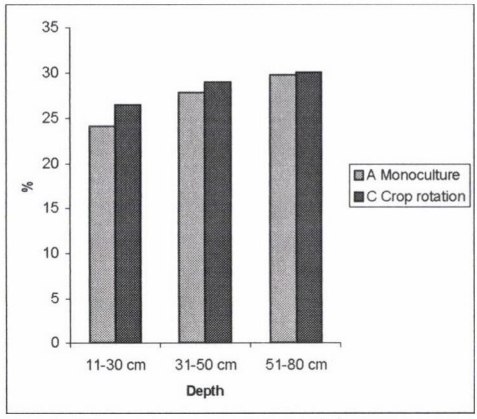

Fig. 2. Water content (w/w) in different cropping systems at three depths (Kompolt, 1997)

Table 2
Water content (w/w) of monoculture (A) and crop rotation (C) at three depths (Kompolt, 1997)

Depth 11–30 cm		Depth 31–50 cm		Depth 51–80 cm	
Plot	Mean	Plot	Mean	Plot	Mean
A/2 10	22.20	C/3 6	26.60	C/3 11	29.02
A/3 9	22.23	A/2 11	26.85	A/3 1	29.51
A/2 11	23.13	A/2 10	26.91	A/3 6	29.55
A/3 6	24.00	A/3 10	27.11	A/2 10	29.60
A/3 10	24.07	A/3 1	27.72	A/2 11	29.61
A/3 1	24.44	A/2 1	27.76	A/3 10	29.72
C/2 6	24.94	A/3 9	28.13	A/2 1	29.73
C/3 6	24.94	C/2 6	28.37	A/3 9	29.73
A/2 9	25.04	A/3 6	28.39	A/2 6	29.81
A/2 6	25.21	A/2 9	28.47	C/3 6	29.92
A/3 11	25.51	A/2 6	28.48	C/2 6	30.11
A/1 1	25.82	A/3 11	28.57	A/2 9	30.16
C/3 9	25.89	C/3 9	29.00	C/2 9	30.22
C/2 9	26.26	C/2 9	29.08	A/3 11	30.26
C/2 10	26.33	C/2 10	29.17	C/2 10	30.26
C/2 11	26.71	C/2 11	29.44	C/3 10	30.28
C/3 10	27.68	C/3 10	29.62	C/3 9	30.29
C/3 11	29.33	C/3 11	30.36	C/2 11	30.40
$LSD_{5\%}$	1.77	$LSD_{5\%}$	1.23	$LSD_{5\%}$	1.02

Below the conventional tillage layer (31–50 cm) there were slight differences in water content, ranging from 26.6–30.36%. Most crop rotation plots contained 29.0–30.36% water, while the monoculture was characterised by 26.85–28.57%; thus different cropping systems can be assumed to have a long-term effect.

In the upper layer (11–30 cm) the tendency described above can also be seen. The highest water contents (25.89–29.33%) were recorded in the same plots. In general, the monoculture plots had lower values (22.20–25.82%), although the significant differences ($LSD_{5\%} = 1.77$) were very limited.

Figure 2 summarises the water content data. At the end of the vegetation period only slight differences existed. However, in the upper layers the crop rotation provided more water.

The results suggest that in this long-term trial the cropping patterns (systems) could be characterized by different soil strength values; however, in practice the picture might be less clear. In the case of compaction problems the positive effect of crop sequences could be an important tool in addition to soil tillage.

Conclusions

1. Long-term crop rotation trials provide a valuable source of information on the effect of cropping patterns on soil strength.

2. Penetration resistance is a good tool in determining changes in mechanical impedance under different cropping conditions.

3. Long-term monocultures may be characterised by higher soil strength values especially below the conventional tillage depth.

4. The effect of long-term cropping patterns (monoculture, crop rotation) on the mechanical impedance of the soil could be observed to a depth of 0–80 cm.

5. In the upper layers (11–50 cm) crop rotation had a positive effect on water content. In the deeper layers no significant difference was found.

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N USE EFFICIENCY AND GRAIN YIELD IN LOWLAND RICE UNDER VARIOUS METHODS OF SOWING AND N MANAGEMENT PRACTICES

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Field experiments were conducted for two years (1995 and 1996) at Tamil Nadu Rice Research Institute, Aduthurai to investigate the effect of different methods of sowing in lowland rice with various N management practices on nitrogen use efficiency (NUE) and yield. The rice was sown using an 8-row drum seeder, sowing in lines and transplanting. N was applied in the form of urea by various methods and splits, with and without green manure. In both the experiments, even though there was no marked difference between the seeding methods, drum seeding resulted in a numerically higher grain yield than the other methods. The grain yield of rice was significantly increased by the application of 100% of the recommended N rate as neem cake blended urea and as urea solution in both the experiments. Higher agronomic efficiency and apparent recovery of N were obtained with the application of 100% of the recommended N as neem cake blended urea or urea solution, and with gypsum and neem cake blended urea application at 75% of the recommended N level.

Key words: N use efficiency, grain yield, lowland rice, methods of sowing

Introduction

In India, rice is currently grown on an area of 42 million ha with a production of about 82 million tonnes. However, at the present level of population growth, India will need an annual increase of not less than 2.5 million tonnes of rice to sustain the present level of self-sufficiency (Pillai, 1996). In Tamil Nadu rice is cultivated over an area of 2.7 million ha with a production of 7.16 million tonnes. During 2000 AD the estimated production will be 8.9 million tonnes with a 4.5% growth rate (Pillai, 1996). The Cauvery Delta Zone is the traditional area of rice cultivation in Tamil Nadu due to the canal irrigation system of the River Cauvery. This zone accounts for about 22.3% of the rice area and 25.3% of the rice production of the state. It is thus known as "The Rice Bowl of Tamil Nadu".

In this zone, rice is cultivated in three distinct seasons, namely *kuruvai* (June–Sept) followed by *thaladi* (Oct–Feb) (in double crop wetlands), and *samba* (Aug–Feb) (in single crop wetlands). *Kuruvai* rice depends solely on Cauvery water from the Mettur Dam, whereas *thaladi* and *samba* rice utilises heavy monsoon rains at the beginning of the season, besides supplementary irrigation with canal water.

Hence, the time of release of the Mettur Dam water for irrigation decides the *kuruvai*, *thaladi* and *samba* rice production. When the water is released late,

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i.e. beyond the scheduled date of June 12th, the harvest of late *kuruvai* rice and the planting of *thaladi/samba* crops overlap, leading to a labour shortage in this zone. This makes it necessary for the farmers to switch over to the direct seeding practice, since this practice has the advantage of eliminating nursery preparation and maintenance, the pulling out of seedlings, transportation and planting, in addition to a shorter crop cycle because of the absence of transplantation shock (Dingtuhn et al., 1987). The grain yield is also comparable to that of the transplanting method and even higher under good management (De Datta, 1986). The different seeding methods followed under puddled conditions are broadcasting sprouted seeds, sowing sprouted seeds in lines and sowing sprouted seeds using a drum seeder. The rice yields achieved with these methods also vary due to variations in the effectiveness of other techniques adopted, especially nutrient and weed management practices.

Among the nutrients, nitrogen (N) generally limits rice production worldwide. Much of the fertiliser N applied to the soil is not utilised fully by the crop and a substantial part is lost either in solution, by leaching as nitrate or ammonia, in gaseous form, as nitrous and nitric oxide gas escaping into the atmosphere, or due to dinitrogen immobilisation in microbial cells or ammonium fixation in the interlattice position of clay minerals. Losses of this costly input cause great concern to agronomists, environmentalists and farmers. Hence there is an increasing need to develop techniques to minimise these losses.

Likewise, legume green manuring and the recycling of crop residues are becoming increasingly important in making today's agriculture sustainable and more efficient. *Sesbania rostrata*, a stem nodule-bearing species introduced recently from the Philippines, is found to meet part of the N requirement and at the same time to maintain soil fertility and productivity. The present study was made keeping all these in mind.

Materials and methods

Field experiments were conducted during the *kuruvai* and *thaladi* seasons of 1995 and 1996 at the Tamil Nadu Rice Research Institute, Aduthurai with the following treatments in a split plot design with three replications:

Main plot (Seeding methods)

S_1 – Transplanting
S_2 – Manual sowing of sprouted seeds in lines
S_3 – Drum seeding of sprouted seeds

Subplot (Nitrogen management)

N_1 – 100% recommended N as prilled urea (1/2 as basal dose + 1/4 at active tillering + 1/4 at panicle initiation)
N_2 – 50% recommended N as prilled urea (1/2 as basal dose + 1/4 at active tillering +1/4 at panicle initiation)
N_3 – 100% recommended N as urea solution (1/3 at early tillering + 2/3 at active tillering)
N_4 – 50% recommended N as urea solution (1/3 at early tillering + 2/3 at active tillering)

N_5 – 100% recommended N as neem cake blended urea (1/2 as basal dose + 1/4 at active tillering + 1/4 at panicle initiation)
N_6 – 50% recommended N as neem cake blended urea (1/2 as basal dose + 1/4 at active tillering + 1/4 at panicle initiation)
N_7 – Green manure application (*Sesbania rostrata*) basally to supply 1/2 of the recommended N + remaining 1/2 of N as prilled urea (1/4 at active tillering + 1/4 at panicle initiation)
N_8 – Control (without N).

In the second experiment the subplot treatments were modified based on the results of Experiment I. The treatments followed were:

Main plot (Seeding methods)

S_1 – Transplanting
S_2 – Manual sowing of sprouted seeds in lines
S_3 – Drum seeding of sprouted seeds

Subplot (Nitrogen management)

N_1 – 100% recommended N as prilled urea (1/2 as basal dose + 1/4 at active tillering + 1/4 at panicle initiation)
N_2 – 100% recommended N as prilled urea (1/3 at early tillering + 1/3 at active tillering + 1/3 at panicle initiation)
N_3 – 100% recommended N as urea solution (1/3 at early tillering + 2/3 at active tillering)
N_4 – 75% recommended N as urea solution (1/3 at early tillering + 2/3 at active tillering)
N_5 – 100% recommended N as neem cake blended urea (1/2 as basal dose + 1/4 at active tillering + 1/4 at panicle initiation)
N_6 – 75% recommended N as prilled urea with gypsum and neem cake blend at a ratio of 5:4:1 (1/3 at active tillering + 2/3 at panicle initiation)
N_7 – Green manure application (*Sesbania rostrata*) basally to supply 1/2 of the recommended N + remaining 1/2 of N as prilled urea (1/4 at active tillering + 1/4 at panicle initiation)
N_8 – Control (without N).

The experimental fields had moderately drained deep clay soil, taxonomically known as Odorthenric chromusterts alluvial clayey (Kalathur series as per Tamil Nadu State Soil Testing Laboratory classification). They were low in available N, medium in available P and K, with a neutral reaction.

Observations on grain yield were recorded and the nitrogen use efficiency (NUE) was calculated as follows.

The agronomic efficiency (AE), i.e. the response in yield per unit N input as indicated by kg of grain per kg of applied N, was computed using the following formula:

$$AE = \frac{\text{Grain yield in fertilised plot (kg ha}^{-1}) - \text{Grain yield in unfertilised plot (kg ha}^{-1})}{\text{Quantity of fertiliser N applied (kg ha}^{-1})}$$

Apparent recovery (AR), which is also known as the recovery fraction, was computed using the formula suggested by Pillai and Vamadevan (1978):

$$\text{Apparent recovery of N (\%)} = \frac{Y_t - Y_0}{N_t} \times 100$$

where:
Y_t – Uptake of N in particular treatment (kg ha^{-1})
Y_0 – Uptake of N in unfertilised plot (kg ha^{-1})
N_t – Quantity of N applied for the treatment (kg ha^{-1}).

Results and discussion

Grain yield

The seeding methods showed no significant influence on the grain yield of rice. However, drum seeding led to a numerically higher grain yield than the other methods (Table 1).

During the first year of the experiment, N management practices significantly increased the grain yield. The application of 100% recommended N as neem cake blended urea (N_5) or as urea solution (N_3) resulted in a significantly higher grain yield than the other treatments in both seasons. This was followed by the integrated application of 50% N as green manure and 50% as prilled urea (N_7) and by the conventional split application of prilled urea (N_1), which were equally effective in producing the next highest grain yield in both seasons.

The best treatment of neem cake blended urea, when applied at 100% of the recommended N level (N_5), increased the grain yield by 15.50 and 16.09% in the *kuruvai* season and by 8.63 and 8.67% in the *thaladi* season over the conventional split application of prilled urea (N_1) and the integrated application of 50% N as green manure and 50% as prilled urea (N_7), respectively. The next best treatment, involving the application of urea solution (N_3), produced 15.1 and 15.7% increase in grain yield during the *kuruvai* season and 3.87 and 3.90% increase in the *thaladi* season over the N_1 and N_7 treatments, respectively.

The interaction effects between seeding methods and N management practices had no significant influence in altering the rice grain yield.

Table 1
Effect of seeding methods and N management practices on grain yield (t/ha)

Treatments	1995–1996		1996–1997	
	Kuruvai	Thaladi	Kuruvai	Thaladi
S_1	5.521	4.825	5.881	5.029
S_2	5.463	4.940	5.823	5.222
S_3	5.630	4.950	5.990	5.266
CD (P = 0.05)	NS	NS	NS	NS
N_1	6.069	5.503	5.379	4.979
N_2	4.472	4.247	5.882	5.081
N_3	6.986	5.716	6.976	5.901
N_4	5.129	4.546	6.409	5.516
N_5	7.010	5.978	7.000	5.898
N_6	5.072	4.543	6.392	5.493
N_7	6.038	5.501	5.648	5.356
N_8	3.529	3.206	3.499	3.154
N	0.5024	0.3690	0.5024	0.2892
S × N	NS	NS	NS	0.5430

NS – Non-significant

During the second year of the experiment, the application of 100% recommended N as urea solution (N_3) or as neem cake blended urea (N_5) had a comparable effect in increasing the grain yield and were significantly superior to the other treatments in both seasons. The application of 75% recommended N as urea solution (N_4) or as gypsum and neem cake blended urea (N_6) were the next best treatments in both the seasons and were on a par with the improved split of 100% recommended N (N_2) in the *kuruvai* season and with the integrated application of green manure and urea each at 50% recommended N (N_7) in the *thaladi* season.

The application of 100% recommended N as urea solution (N_3) or as neem cake blended urea (N_5) and of 75% recommended N as urea solution (N_4) or as gypsum and neem cake blended urea (N_6) led to 29.68, 30.14, 19.15 and 18.83% increases in grain yield, respectively, in the *kuruvai* season and 18.51, 18.45, 10.78 and 10.32% increases, respectively, in the *thaladi* season compared to the conventional split application of 100% recommended N (N_1).

The interaction effects between seeding methods and N management practices showed a significant influence in the *thaladi* season, but not in the *kuruvai* season.

Neem cake blended urea and the deep placement of urea solution are subject to lower N losses. Dutta et al. (1995) also confirmed the positive influence of neem cake blended urea in increasing the grain yield of rice due to the nitrification inhibition activity of the neem cake. The yield increase under improved split application over conventional split application might be due to lower losses of N due to the timely supply of nutrients. The integrated application of green manure and prilled urea at 100% recommended N showed a comparable effect with 100% N broadcast as prilled urea. The partial substitution of green manure for the inorganic N was the reason for this effect, as reported by Jayapaul et al. (1995) and Pandey et al. (1995).

A comparison of the yield data for the two seasons showed a higher yield of rice in the *kuruvai* season than in the *thaladi* season. This could be explained by the favourable weather factors prevailing in the *kuruvai* season compared with the *thaladi* season. The prevalence of higher maximum and minimum temperatures and solar radiation positively influenced the yield of rice in the *kuruvai* season, while the low solar radiation and maximum temperature, the high relative humidity and the occurrence of rainfall between panicle initiation and the 50% flowering stage resulted in the lower yield during the *samba/thaladi* season (Jayaraman et al., 1993).

Effect of treatments on Nitrogen Use Efficiency

The NUE was not markedly influenced by the different methods of seeding (Table 2).

Table 2
Effect of seeding methods and N management practices on NUE*

Treatment	Agronomic efficiency (kg grain/kg N)				Apparent recovery (%)			
	1995–1996		1996–1997		1995–1996		1996–1997	
	Kuruvai	Thaladi	Kuruvai	Thaladi	Kuruvai	Thaladi	Kuruvai	Thaladi
S_1	20.9	14.7	21.5	13.7	39.3	42.0	36.3	33.4
S_2	16.2	13.1	17.0	13.9	39.0	41.8	36.7	33.8
S_3	24.0	16.3	24.4	16.6	39.2	42.1	36.5	34.0
N_1	20.3	17.5	15.0	12.2	36.4	37.8	34.5	30.8
N_2	16.2	14.0	19.1	12.8	45.7	53.0	35.3	32.4
N_3	27.6	16.7	27.8	18.3	43.1	43.4	43.4	43.2
N_4	25.6	17.8	31.0	21.0	53.9	59.8	50.2	44.0
N_5	27.8	18.4	28.0	18.3	43.4	43.6	44.4	43.1
N_6	25.6	17.8	29.6	20.8	54.2	60.1	48.9	44.6
N_7	20.0	15.3	17.2	14.7	36.7	38.0	35.3	31.8
N_8	–	–	–	–	–	–	–	–

* Data not statistically analysed

Agronomic efficiency

During the first year of the experiment the agronomic efficiency was highly improved by the application of 100% recommended N as neem cake blended urea both in the *kuruvai* and *thaladi* seasons. Higher agronomic efficency of 27.8 and 18.4 kg grain per kg of N applied was obtained with 100% recommended N as neem cake blended urea in the *kuruvai* and *thaladi* seasons, respectively. The deep placement of urea solution at 100% recommended N (N_3) in the *kuruvai* season and at 50% recommended N (N_4) in the *thaladi* season were the next best treatments in recording higher agronomic efficiency.

During the second year, agronomic efficiency was highly improved by the application of urea solution at 75% recommended N (N_4) followed by gypsum and neem cake blended urea at 75% recommended N (N_6) in both the *kuruvai* and *thaladi* seasons. Higher agronomic efficiency of 31.0 and 21.0 kg grain per kg of N applied was obtained with the urea solution at 75% recommended N level in the *kuruvai* and *thaladi* seasons, respectively. The application of 100% recommended N as urea solution (N_3) or as neem cake blended urea (N_5) were the next best treatments in recording higher agronomic efficiency in both seasons.

Higher AE at a lower level of N application than at a higher level was also reported by Devasenapathy (1994). The N release pattern in these treatments may have been well matched with the N requirement of the rice crop, resulting in higher AE (Schnier et al., 1990).

Apparent Recovery

In the first experiment, the application of neem cake blended urea at 50% recommended N resulted in higher apparent recovery of 54.2 and 60.1% in the *kuruvai* and *thaladi* seasons, respectively, than all the other N management practices. In both seasons the application of urea solution at 50% recommended N was the next best treatment. Among the 100% recommended N application treatments, neem cake blended urea (N_5) gave the highest apparent recovery percentages of 43.4 and 43.6 in the *kuruvai* and *thaladi* seasons, respectively, followed by urea solution (N_3). The integrated application of 50% N as green manure and 50% as prilled urea (N_7) and the conventional split application of prilled urea (N_1) were comparable in both seasons.

During the second year, a higher apparent recovery of 50.2% was achieved by treatment with urea solution at 75% recommended N (N_4) in the *kuruvai* season, while the application of 75% recommended N as gypsum and neem cake blended urea (N_6) led to a higher value (44.6%) in the *thaladi* season. The application of 100% recommended N as neem cake blended urea (N_5) or as urea solution (N_3) were the next best treatments in recording higher apparent recovery of N.

Gypsum blended urea maintains a higher NH_4-N content in the soil due to the slow mineralisation and nitrification processes and also reduces the NO_3-N content in flooded rice soil due to the inhibitory effect of calcium on NH_4 transformation (Saravanan, 1996). AR was higher at lower levels of N than at higher levels. The increased availability of N with reduced N losses in these treatments might have increased the AR. Higher N losses with less N recovery due to ammonia volatilisation and denitrification might have resulted in the low AR under conventional methods of fertiliser application (Schnier et al., 1988).

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EVALUATION OF INTERACTION BETWEEN PLANT DENSITY AND SOIL CULTIVATION IN MAIZE PRODUCTION

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The effect of plant density and soil cultivation on maize yields was evaluated each year between 1989 and 1994 and over the average of the experimental years in a long-term experiment set up in the Látókép field nursery of Debrecen University of Agricultural Sciences.

The results of variance analysis in the experimental years and over the average of the years indicated a close correlation between soil cultivation variants and plant density. In the case of autumn ploughing a plant density of 70–80 thousand per hectare was the most favourable, with the lower figure in dry years and the higher plant density in years with average rainfall. Especially in the case of minimum tillage, plant densities greater than 60 thousand led to yield losses. In all the plant density variants the yield was higher in the minimum tillage treatment than in the case of spring ploughing: even in the 80 thousand-plants/hectare variant the yield surplus was almost a ton. In dry years the disadvantages of spring ploughing were clearly perceptible in the yield figures. Spring ploughing was not only unable to provide a satisfactory seedbed for maize germination and uniform emergence, but also led to water loss, which inhibited the uniform development of the plant stand during the critical summer period. If spring ploughing is unavoidable, a maximum plant density of 70 thousand is recommended, with lower plant densities in dry years.

Variance analysis of the results indicated that the effect of plant density and the interaction between plant density and soil cultivation varied according to the water supplies, but were significant in all the experimental years and over the average of the six years. The only exception was 1991, when rainfall supplies were favourable and the yields did not differ significantly from each other. In dry years lower plant densities gave better results for all three soil cultivation methods (autumn or spring ploughing and minimum tillage). Higher plant densities increased the risks of maize production and reduced efficiency.

Key words: tillage, plant density, maize yield

Introduction

According to Holliday's (1960) research results there is a basic biological correlation between yield and plant density. In the case of plants where the economically useful yield is provided by the plant's reproductive parts (grain yield), the correlation between the yield and the plant density can be characterized by a parabolic function. Maize belongs to this group. If the useful yield is provided by the vegetative parts of the plant the correlation can be described by an asymptotic (saturation) function. The correlation is also asymptotic between the gross dry matter production (biomass) and the plant

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density. Generally, maize shows a very definite decrease in yield at high density and represents one of the most extreme forms of parabolic correlation. The biological correlation mentioned above has been described using various equations and differences in its biological interpretability have also been noted (Willey and Heath, 1970; Duncan, 1984). Russell (1968) compared the yield of maize hybrids in six different environments, using four plant densities. Averaged over the densities the yields were almost the same, but the reactions differed significantly. The average yield of maize in the USA has increased more than fourfold since the introduction of maize hybrids in 1930. Plant density, and hybrids possessing the genetic potential to give large yields at high density were important components in this yield increase. Duvick (1984) verified that plant density was an important factor in the yield increase of hybrids introduced in the 1970s and 1980s. Russell (1984) compared 4 single cross hybrids each from six ten-year periods between 1930 and 1980, carrying out the evaluation at three plant densities (30,000, 47,800 and 64,500 plant/ha) in four different areas. The yield responses in the different periods were quite distinct. The hybrids of the 40s had the greatest negative response to plant density; the hybrids of the 70s and 80s had a similar positive reaction, but the hybrids of the 80s gave the greatest yield average with an increase in the plant density. On the basis of the above could be stated that both in Duvick's (1984) and Russell's (1984) experiments the hybrid × plant density interaction was strongly significant. Tollenaar (1989) proved that the optimum plant density of hybrids in the Corn Belt increased at a rate of 0.97 plants/m^2/10 years between 1950 and 1980. In France, the linear increase for hybrids with a late breeding season was similar, being 0.96 plants/m^2/10 years (Derieux et al., 1987).

In Hungary, the first plant density experiments on maize were carried out by Berzsenyi-Janosits (1953) and I'so (1966). Győrffy (1979) continued the work begun by I'so and revealed the most important factors in the plant density effect and how these were connected with soil cultivation. He pointed out that the optimum plant density for maize hybrids was 35–40 thousand per hectare in the fifties, 50 thousand per hectare in the sixties and 55–60 thousand in the seventies. He established the fact that the optimum plant density depends on the hybrid sown, on the precipitation conditions of the region, on the water management of the soil and on the nutrient supply level. Bajai (1966), Nunez and Kampraht (1969) and Németh (1997) demonstrated a correlation between the yield of maize and the growing area per plant. In Hungary, Győrffy (1979) was the first to point out that there are differences in the optimum plant density range for different hybrids. A distinction can be made between hybrids with narrow and wide plant density optima. Over the last 15 years farmers in Hungary have favoured the latter. A number of correlated factors (tillage, fertilization and irrigation) may have an effect on the plant density responses of the hybrids. The latest research results indicate that the optimum plant density of the hybrids does not depend solely on the length of the growing season but also on the genotype (Allison, 1969; Bunting, 1971; Nagy and Bodnár, 1986; Berzsenyi et al., 1994; Széll, 1994; Nagy, 1995). Research carried out by Lap and Berzsenyi (1993) at

Martonvásár showed significant plant density interactions and confirmed the considerable modifying effect of the year. According to this research, growth analysis (Petr et al., 1985) is a useful and important method for quantifying the individual and combined effects of the experimental treatments on the growth of maize. After examining the effect of the given year they concluded that in wet years the decrease in dry matter production was greater in treatments where no fertilization was used. Without fertilization the grain yield of maize decreased significantly in wet years, at above 60,000 plants/ha. In dry years, however, an increase in plant density from as low a level as 30,000 plants/ha did not result in any yield increase. The experimental results of Csaláné (1992) were in agreement with the results of other research institutes, all indicating that an increase in plant density was an important tool in increasing yields over the past 20 years. At the same time attention is drawn to the fact that both too high and too low plant densities may result in yield decreases. Németh et al. (1982) reported on the results of 10-year research carried out at the Cereal Research Institute in Szeged and concluded that genetic progress can be measured not only by the increased productivity of the hybrids but also by their plant density reactions. Hybrids with a wider plant density interval were less sensitive to technological errors. On examining the plant density reactions of new hybrids from Martonvásár, Varga (1991) concluded that the results of plant density experiments provided a good source of information on the growing area needs of the hybrid, and could thus be of assistance to farmers in choosing the best variety and the correct plant density.

On the basis of research, carried out in Hungary, Győrffy (1979) pointed out that one of the most important ways of increasing yield is for all the plants to have nearly the same size of growing area. In achieving a homogeneous crop stand, good tillage, soil preparation, an even ploughing depth, good water and nutrient supplies, soil sterilization and the quality of the seed, all play an important role (Csizmazia, 1997; Jolánkai et al., 1997; Rajkai et al., 1997). Plant density experiments set up in different research institutes and universities in Hungary over the past 10–15 years have contributed significantly to our knowledge on the plant density responses of maize hybrids and to determining the optimum plant density required to ensure outstanding yields.

Materials and methods

At the experimental farm of the Department of Crop Science and Land Use of Debrecen Agricultural University in Látókép, the effects of crop cultivation factors on the yield of maize are studied on a chernozem soil with lime deposits. The research is based on the scientific results of Győrffy (1979). A polyfactorial long-term experiment has been set up for the evaluation of the effects of plant density and soil cultivation. The maize hybrids examined were Volga SC, Pannónia SC and Dekalb 524 SC. The size of each tillage block was 4320 m^2. In planning the soil cultivation methods, the goal was to have a difference in the depth of cultivation as well as in the quality of soil preparation. No ploughing was used in one method, while the depth of general purpose cultivation was 12 cm. Autumn ploughing was carried out to a depth of 27 cm and spring

ploughing, prior to sowing, to a depth of 22 cm. The size of each tillage block was 270 m^2 ($4 \times 270 = 1080$ m^2).

The treatments were as follows:

Cultivation	T_1 = without ploughing (12 cm)
	T_2 = spring ploughing (22 cm)
	T_3 = autumn ploughing (27 cm)
Plant density	N_1 = 60,000 plants/ha
	N_2 = 70,000 plants/ha
	N_3 = 80,000 plants/ha
	N_4 = 90,000 plants/ha

Soil characteristics

The soil of the experimental area was a calcareous chernozem with a lowland loess basic layer. It had average N and P supplies and a high K supply (humus content = 2.8–3.0%, total N = 0.14–0.18%, AL-P_2O_5 = 130–200 mg/kg, AL-K_2O = 240–280 mg/kg). The depth of the humus layer was 70–90 cm. The pH value (KCl) was 6.2 and the liquid limit according to Arany 43. No microelement deficiency could be detected. The groundwater level was between 6–8 m. The minimum water holding capacity (WHC_{min}) of the soil was 27–29 volume %. The water storage capacity of the soil was 275 mm in the 0–100 cm profile and 265 mm in the 100–200 cm profile. The dispensable field capacity (FC) was 157 mm and 150 mm in the 0–100 and 100–200 cm profiles, respectively.

Weather characteristics

Over the period examined, precipitation in Debrecen was unfavourable (drought) for maize in 1990, 1992 and 1994, while in 1989, 1991 and 1993 the rainfall conditions were average. In order to ensure the reliability of the evaluation up-to-date experimental design methods were applied in planning the research project, using an improved version of the method theoretically proved by Box and Wilson (1951). In the evaluation of the experimental data, variance analysis was used with the desegregation of the variance components (Sváb, 1981; John, 1971; Winer, 1971). The method of Box and Cox (1964) was used to stabilize the variance of the blocks. For the desegregation of the variance components the method of "maximum likelihood" was applied. On the basis of a suggestion made by Huzsvai (1994) a mixed, fix-random model was compiled to evaluate the effects. Within the real replications the hybrids were also considered as replications since they showed differences over the average of five years. The evaluation was done on an IBM 486 DX computer with the 1988 version of the BMDP Statistical Software.

Results and discussion

The effects of plant density and soil cultivation on the yield of maize were evaluated annually between 1989 and 1994, as well as over the average of the years, using a long-term experiment conducted at the experimental farm of Debrecen Agricultural University.

According to the results of variance analysis the plant density effect was significant in 5 of the 6 years examined. The exception was the year 1991, when the yield of the different plant density variants did not differ significantly from each other (Table 1).

In 1989, the most favourable year for maize, the greatest yield was obtained after autumn ploughing (11.92 t/ha) in a crop stand with 80 thousand plants/ha. After spring ploughing the best plant density was 70–80 thousand and where no ploughing was used the optimum was even less, 70 thousand plants/ha.

Table 1
Effect of plant density and soil cultivation on the yield (Debrecen, 1989–1994)

Soil cultivation	Plant density/ha	Yield, t/ha						
		1989	1990	1991	1992	1993	1994	*Average*
Autumn ploughing	60,000	10.63	9.64	11.06	6.78	8.70	6.81	*8.94*
	70,000	11.66	9.50	11.04	6.89	9.64	7.51	*9.37*
	80,000	11.92	9.05	11.22	6.60	8.85	7.77	*9.24*
	90,000	11.27	8.64	10.85	6.20	8.26	6.76	*8.66*
	Average	*11.37*	*9.21*	*11.04*	*6.62*	*8.86*	*7.21*	*9.05*
Spring ploughing	60,000	10.15	9.71	10.43	6.30	7.32	6.31	*8.37*
	70,000	10.56	9.69	11.31	6.36	7.59	6.46	*8.66*
	80,000	10.79	9.15	11.33	6.06	7.48	6.11	*8.49*
	90,000	10.07	8.53	11.51	5.93	7.24	5.81	*8.18*
	Average	*10.39*	*9.27*	*11.15*	*6.16*	*7.41*	*6.17*	*8.43*
Minimum tillage	60,000	9.85	10.03	10.45	6.29	6.23	6.94	*8.30*
	70,000	10.35	9.70	10.18	6.39	6.29	7.19	*8.35*
	80,000	10.23	9.03	9.88	6.03	5.80	7.15	*8.02*
	90,000	9.60	8.23	9.71	5.64	5.62	6.63	*7.57*
	Average	*10.01*	*9.25*	*10.06*	*6.09*	*5.99*	*6.98*	*8.06*
$LSD_{5\%}$	Plant density (Pd)	0.12	0.11	ns	0.13	0.10	0.07	0.08
	Soil cultivation (Sc)	0.14	ns	0.24	0.14	0.10	0.08	0.60
	Pd × Sc	0.20	0.18	ns	0.29	0.17	0.13	0.14

ns = non-significant

During the drought in 1990, a plant density of 60 thousand plants per hectare gave the greatest yield after both autumn and spring ploughing, as well as in the method where no ploughing was used. In all three tillage methods increased plant densities decreased the yield. The yield was the greatest (10.03 t/ha) in the method where no ploughing was used. In autumn and spring ploughing the yields were lower and did not differ significantly from each other.

In 1991, owing to the favourable natural water supply, the plant density effects were not significant. A comparison of the soil preparatory methods showed that the yields after spring and autumn ploughing were nearly the same and did not differ significantly. At the same time, in the method where no ploughing was used the yields were significantly less by a tonne.

In 1992, the growing season was dry. During the winter term, precipitation was extremely low, being 151 mm less than the 50-year average. Of the years examined, the yield was the lowest in 1992 (6.16–6.62 t/ha) after both spring and autumn ploughing. In all three tillage methods, as in 1990, the lowest plant density (60 thousand/ha) was the most favourable. The 70 thousand plants/ha variants did not produce significantly greater yields compared to 60 thousand plants/ha. In the 80 and 90 thousand plants per hectare variants – in all three tillage methods – the yield decreased significantly compared to lower plant densities.

In 1993, precipitation was 50 mm lower during the growing season but there was three and a half times as much precipitation from the harvest of the forecrop until sowing compared to the previous year. The more favourable water supply was shown in the yield after autumn and spring ploughing. In the method where no ploughing was used, there was no significant difference between the two years; more precipitation did not increase the yield. However, after autumn ploughing the yield was 2.24 tons more per hectare averaged over plant densities, and 1.25 tons greater after spring ploughing than in 1992, when the water supply was less favourable. In 1993 the plant density effects were significant (Table 2). After spring and autumn ploughing the 70 thousand plants per hectare variants gave higher yields (7.59–9.64 t/ha). In the method where no ploughing was used, the 60 and 70 thousand plants/ha variants did not differ significantly, while the greater plant densities (80–90 thousand plants/ha) gave significantly lower yields.

In 1994, the water shortage was similar to that in 1992, but in the critical July–August period the drought was even more severe. Owing to this, the greatest yield was obtained in the method where no ploughing was used at 60 thousand/ha plant density. Plant density, tillage and the correlation of these were all significant (Table 3). In all the plant density variants, the yield was more favourable when no ploughing was used, even in the 80 thousand plants/ha variant, where the surplus yield was one tonne compared to spring ploughing. In this critically dry year, the drawback of spring ploughing was measurable in the yield (Fig. 1). Spring ploughing was unable to provide a good seedbed for germination and uniform emergence, and the water loss caused by soil preparation also hindered the uniform development of the crop stand in the critical summer period.

Table 2
Results of variance analysis (Debrecen, 1993)

Source of variance	SQ	FG	MQ	F	α-error
Soil cultivation	1195.32834	2	597.66417	1763.28	≈0.0000
Plant density	69.57150	3	23.19050	83.39	≈0.0000
Plant density × soil cultivation	30.05019	6	5.00836	18.01	≈0.0000
Deviation	45.05355	162	0.27811		

Table 3
Results of variance analysis (Debrecen, 1994)

Source of variance	SQ	FG	MQ	F	α-error
Soil cultivation	290.31454	2	145.15727	383.16	≈0.0000
Plant density	99.78154	3	33.26051	130.76	≈0.0000
Plant density × soil cultivation	43.55508	6	7.25918	28.54	≈0.0000
Deviation	68.67966	162	0.25437		

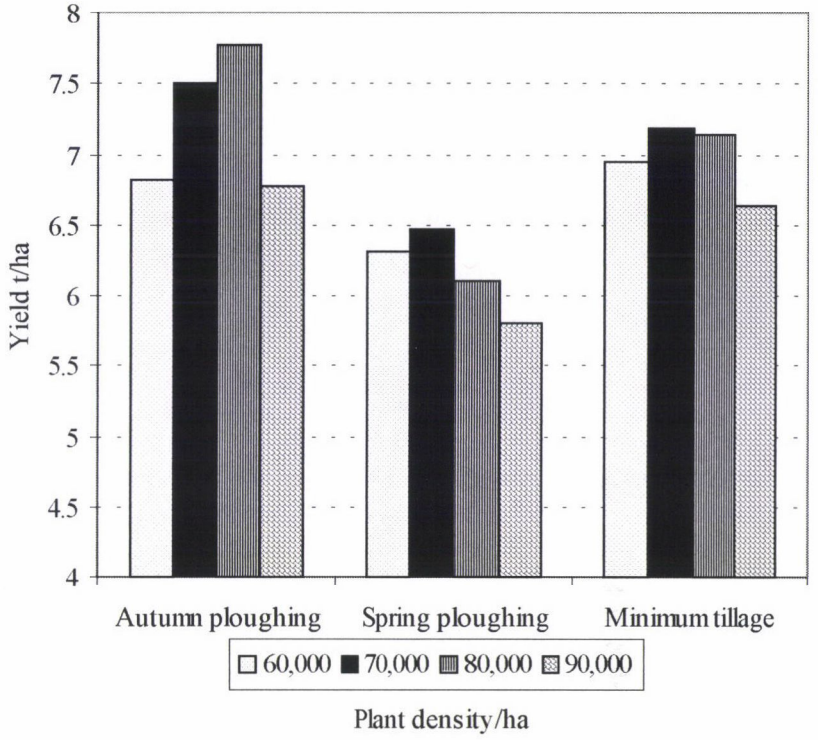

Fig. 1. Effect of plant density and soil cultivation on the maize yield (Debrecen, 1994)

According to the results of variance analysis, in the years examined and over the average of the years, the tillage methods showed a strong correlation with plant density. After autumn ploughing 70–80 thousand plants/ha was the most favourable, the lower density being more satisfactory in years of drought, and the greater plant density in years with average precipitation. Especially in the method where no ploughing was used, plant densities exceeding 60 thousand caused a loss of yield. According to the experimental results, if spring ploughing is unavoidable a maximum of 70 thousand plants per hectare should be sown, while even lower plant densities are recommended in years of drought using this method of preparation.

According to variance analysis the plant density effect and the plant density × soil cultivation correlation differred depending on the water supply, but were significant in all the years examined and over the average of six years. The only exception was 1991, when the yields did not differ significantly owing to the favourable precipitation. In years of drought, lower plant densities were significantly better in all three soil preparatory methods (autumn, spring, and no ploughing). Greater plant densities increased the risks of maize production and decreased its efficiency.

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Short communication

JUSTIFIABILITY OF FLOWERSTEM TRIMMING IN SUGAR BEET

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A three-year field trial was established in order to study the effects of flowerstem trimming on seed yields and seed size in sugar beet. Both the first treatment (cutting the stem down to 15 cm) and the second treatment (cutting down to a height of 30 cm) produced a significant increase in seed yield compared with plants whose flowerstems had not been trimmed (control treatment). In all the hybrids studied from both treatments the proportion of smaller seeds (< 3.5 mm) increased relative to the control.

Key words: sugar beet seed, flowerstem trimming, seed yield, seed fractions

Introduction

Flowerstem trimming in sugar beet grown for seed production purposes has never been practiced in Yugoslavia, although this technique has long been known across the world.

Vlatković (1991) has shown that if sugar beet flowerstems are trimmed down to 30–35 cm in mid-May, when the plants are usually about 60 cm tall and the primary branching begins, seed yields will increase significantly in relation to untrimmed plants.

When sugar beets are grown for seeds by means of direct sowing, the result is usually a tall primary stem with secondary branching in the uppermost third of the plant. Direct sowing is typical of seed sugar beet growing in North America and countries like the UK, Hungary and Yugoslavia, all of which have a considerably larger number of plants per hectare (Bornscheuer et al., 1993). A special French method for increasing seed yield, known as Broyage, consists of cutting off the flowerstem 5 cm above the crown at the start of intensive growth.

Flowerstem trimming promotes intensive branching from axillary buds, leading to the expression of desirable traits, such as reduced flowerstem height (by about 50 cm), more uniform flowering and seed ripening, and, most importantly, increased seed yield (Leguillette, 1987).

In both plants sown directly and those planted using stecklings, the trimming of the flowerstem at a height of 12–20 cm during early development (a method used in the U.S.) will lead to the uniform development of this part of the plant (Scott, 1969, cit. Dobrotvorceva, 1975).

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Bornscheuer et al. (1993) reported that flowerstem trimming increased seed yield and seed viability. The largest seed fractions (4.0–4.5 and 4.5–5.0 mm) have the highest germination percentage (Milošević et al., 1992). Leguillette (1987) established that flowerstem trimming increased seed size and hence germination percentage. Flowerstem trimming is particularly widespread in France, where it has a positive effect on seed yield levels (Nardi, 1991).

Materials and methods

Used in the study were three domestic sugar beet hybrids: NS-HY-11, Nera and Norma, and three joint German-Yugoslav ones: Nomega, Delta and Dana. The experimental design was a randomized block with three replications and the trial was carried out at the Institute's experiment field at Srbobran. The basic plot size was 10 m^2 and the experiment lasted three years. There were three treatments: i) flowerstem trimming at a height of 15 cm; ii) trimming at 30 cm; and iii) no trimming (control). The flowerstems were cut at the beginning of intensive growth. The seed from all treatments was assorted according to size using <3.50; 3.50–4.50; and 4.50–6.00 mm sieves. The yield of seed after primary processing was determined with 2.0–6.0 mm sieves.

The objective of the study was to determine how flowerstem trimming at two different heights at the stage of intensive growth affects seed yield and size in the six aforementioned hybrids of sugar beet.

Results

In NS-HY-11, Nera and Norma, both trimming heights produced significant differences in the yield of cleaned seed relative to the control (Table 1). In the other three hybrids, however, the difference was present only when the flowerstems were cut at 30 cm. At this trimming height, Nomega gave the highest and Norma the lowest seed yield (1.80 t/ha 1.18 t/ha, respectively).

The yields of cleaned seed were significantly affected by the hybrid (A), trimming (B) and the year (C) (Table 2). The effects of the hybrid/trimming (AB), hybrid/year (AC) and trimming/year (BC) interactions were also significant.

Table 1
Effect of flowerstem trimming in sugar beet on seed yield (t/ha)

Hybrids	Trimming			
	Control 0 cm	First variant 15 cm	Second variant 30 cm	Average
Nomega	1.70	1.66	1.81	1.72
Delta	1.63	1.60	1.73	1.65
Dana	1.53	1.30	1.62	1.48
NS-Hy-11	1.20	1.40	1.50	1.37
Nera	1.30	1.55	1.50	1.45
Norma	1.04	1.10	1.18	1.11
Average	1.40	1.43	1.55	

LSD	Hybrids	Trimming	Hybrids/Trimming	Hybrids/Year
0.05	0.42	0.03	0.07	0.07
0.05	0.55	0.04	0.09	0.09

Table 2
Analysis of variance

Sources of variation	Degrees of freedom	Mean Square	F-value
Replication	2	0.010	1.667
Factor A (hybrids)	5	1.268	211.33**
Factor B (trimming)	2	0.355	59.17**
Facotr C (years)	10	0.079	13.17**
AB	2	0.260	43.33**
AC	10	0.024	4.00**
BC	4	0.016	2.67*
ABC	20	0.009	1.50
Error	106	0.006	–
Total	161	9.484	–

Flowerstem trimming affected seed size only in the case of the smallest seed fraction (<3.5 mm) (Fig. 1). The hybrid Delta had an increased percentage of <3.5 mm seeds in both treatments relative to the control.

In the first treatment (i), the hybrid Dana had a higher percentage of the smallest seeds compared with the second treatment (ii) and the control (iii). All the hybrids had a higher percentage of smaller seeds, but there were no significant differences relative to the control. In the case of the larger seed fractions (3.50–4.50 and 4.50–6.00 mm), flowerstem trimming neither increased nor decreased the seed yield (Fig. 1).

Discussion

Sugar beet intended for seed production purposes grows very rapidly at the flowerstem and flower bud formation stage. When conditions for the growth and development of seed sugar beets are favourable, the rate at which their flowerstems grow can be as high as 5–10 cm a day (Stanaćev. 1979).

In the production of seed sugar beet. flowerstem trimming can be applied as a crop tending measure that leads to an increase in the yield of cleaned seed (Dobrotvorceva, 1975; Leguillette, 1987; Cooke and Scott, 1993). The trimming height is very important as a factor that may affect the yield of cleaned seed. Montari et al. (1983) report seed yield losses after flowerstem trimming at 5 cm. By contrast the present results indicate that flowerstem trimming can produce significant differences in seed yield.

By cutting off the flowerstems at 15 and 30 cm. it was hoped to determine which particular trimming height could be used to increase cleaned seed yields under Yugoslavian conditions. In all the hybrids. trimming at 30 cm produced significant increases in seed yield (Table 1). Seed yield levels also depended significantly on the hybrid and the environmental conditions. i.e. the year (Table 2).

Flowerstem trimming affected seed size only in the case of the smallest seeds (<3.5 mm) although the differences were not significant in all the hybrids.

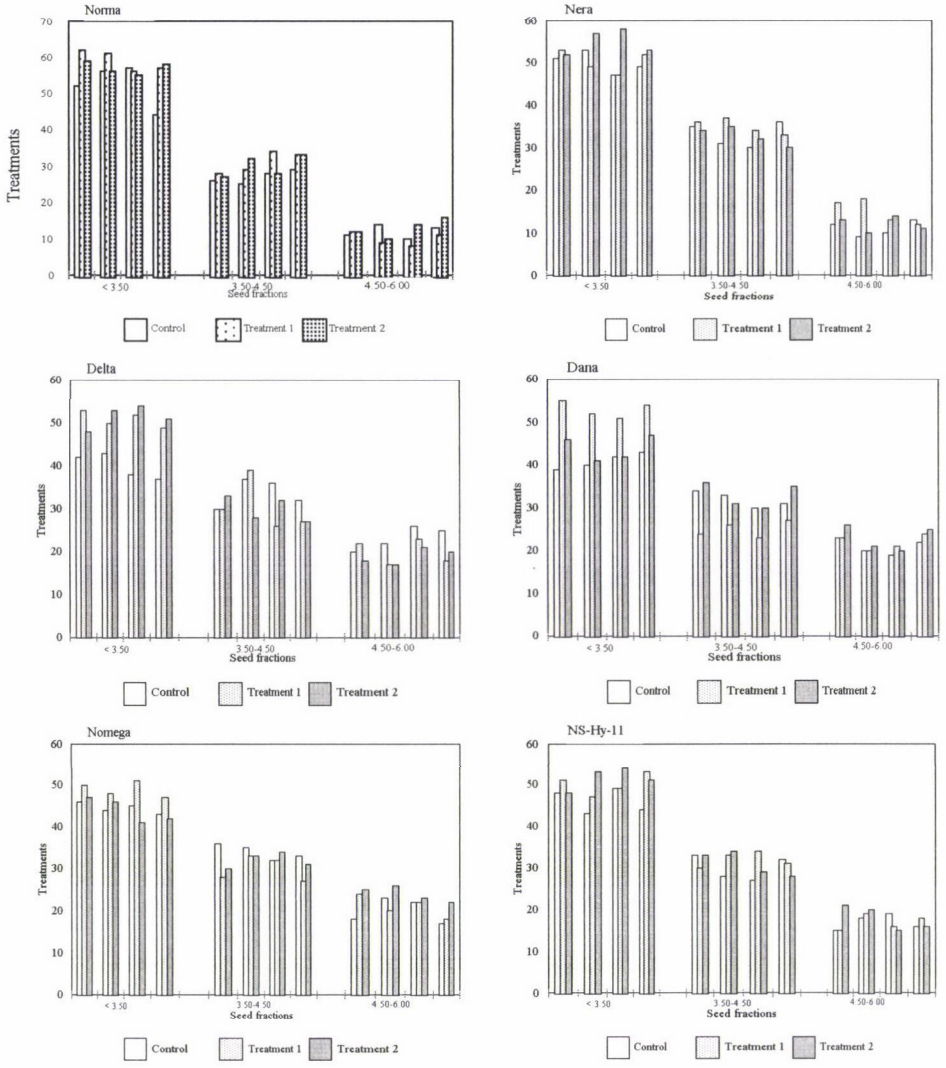

Fig. 1. Effect of flowerstem trimming on seed size in various sugar beet hybrids

The hybrid Nomega produced the highest yield in all three treatments (Table 1). Nomega belongs to the group of hybrids with an average sugar content and is characterized by intensive growth, robustness, resistance to bolting. leaf diseases (*Cercospora*) and most importantly a well-balanced relationship between yield and quality.

Overall. the second best variety in terms of seed yield was the triploid hybrid variety Delta. This hybrid has a high tolerance to *Cercospora* as well as excellent adaptability.

The smallest seed yields in the entire trial were produced by the hybrid Norma, which is a hybrid with an average sugar content characterized by uniform root shape and size (Kovačev, 1993).

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Short communication

EVALUATION OF VARIETAL RESPONSE OF SOYBEAN (*GLYCINE MAX.* L. MERRILL) TO NITROGEN (N) FERTILIZATION IN TASHKENT, CENTRAL ASIA

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A field experiment was conducted under the continental climatic conditions of Tashkent in Central Asia to evaluate the response of three varieties of soybean, an atmospheric nitrogen-fixing legume, to nitrogen (N) fertilization on a phosphorus-potassium background. The rates of N fertilizer were 0, 50, 100, 150, 200 and 250 kg·ha^{-1} N as ammonium sulphate with a phosphorus-potassium fertilizer rate of 120 kg·ha^{-1} P_2O_5 as single super phosphate and 90 kg·ha^{-1} K_2O as muriate of potash. The growth parameters of the varieties Yuldus, Dustlik and Uzbekskaya 2 responded positively ($P \leq 0.05$) to N fertilizer application. Optimum plant height for all the varieties was recorded at the 150 kg N·ha^{-1} level. The number of leaves increased linearly with the increase in the N rate but declined significantly at N rates exceeding 100 kg in all the varieties. The plant density of the varieties was linearly influenced by increased N application but significantly ($P \leq 0.05$) reduced at the 250 kg N·ha^{-1} level. Optimum pod yield was obtained at 150 kg N·ha^{-1} rate. Similarly, the grain yield was significantly increased by an increase in N rates, with optimum yields of 3.1–4.0 tons ha^{-1} at the 100 and 150 kg) levels. The optimum N requirements for the three varieties of soybean was between 100–150 kg N·ha^{-1} under the experimental conditions of Tashkent, Central Asia.

Key words: growth, nitrogen fertilizer management, soybean, varietal response, yield

Introduction

Soybean (*Glycine max.* L. Merrill) is a nitrogen-fixing, protein-rich leguminous crop cultivated for centuries in its centre of origin (North-East China) and in South-East Asia (Kogan, 1981). At the same time, the protein efficiency in the diets of millions of people in tropical and sub-tropical climates is a critical problem in recent times (N. A. S., 1984). A meaningful solution to this problem will depend largely on increased yields of protein-rich leguminous crops such as soybean. In feeding the growing population and in providing greater food security in developing countries, fertilizers have played a leading role in the past and will continue to do so in the future (Baanante et al., 1989; Vlek, 1989). This is particularly true in view of the fact that the areas likely to be brought under cultivation will need organic and inorganic fertilizers to maintain

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their yield potential, because the soils of most of the areas are naturally fragile and new crop technologies, such as improved varieties, are fertilizer-intensive, and without fertilizers their yield potentials are lower than those of traditional varieties.

In order to obtain the full genetic potential of new varieties of soybean adapted to Uzbekistan they must be grown under improved conditions of soil fertility and crop management. This paper describes the optimum requirements of three newly bred varieties of soybean to N fertilization in the continental climate of Tashkent in Central Asia.

Materials and methods

A field experiment was conducted in 1986 on the experimental field of the Department of Crop Science in the Experimental Station of the Tashkent Agricultural Institute, Tashkent, Uzbekistan in Central Asia. The experimental station was located 6 km from Tashkent City and situated on the left bank of the 'Bos-su' Canal 408 m above sea level. The plot relief was uneven and slightly hilly, sloping towards the 'Salar' Canal. The soil was clayed chernozem with a slightly less than moderate level of NPK availability and poor infiltration. The soil contained naturally occurring *Rhizobia*. The underground water table was at a depth of 5–6 m. The amount of rainfall was 200–541 mm/year.

Seeds of three new cultivated varieties of soybean: Yuldus, Dustlik and Uzbekskaya-2 were obtained from the Department of Leguminous Crops of the Rice Research Institute of Uzbekistan.

A one-factor experiment fitted into a randomized complete block design (RCBD) and replicated 4 times was used. The plot size was 50 m^2 and 20 plants were observed per treatment per replication. The seed rate was 80 kg/ha using a 60 × 20 × 1 spacing design. The treatments were N_0, N_{50}, N_{100}, N_{150}, N_{200} and N_{250} kg ha^{-1} derived from ammonium sulphate fertilizer, on a 120 kg ha^{-1} P_2O_5 and 90 kg ha^{-1} K_2O (a.i.) background. The plants were harvested manually by hand at the brown stage and before the shattering of the pods. The data were subjected to analysis of variance (ANOVA).

Results

Observations on plant height showed that all the tested varieties responded positively to a progressive increase in N rates, attaining an optimum height of between 69.3 cm and 91.0 cm at the N_{150} level. However, there was a growth decline at the higher N_{200} and N_{250} rates in all the varieties (Table 1). The stem girth of all the varieties remained the same at different N rates (Table 2).

Table 1
Effect of different N rates on plant height (cm)

Variety	N rate					
	N_0	N_{50}	N_{100}	N_{150}	N_{200}	N_{250}
Yuldus	68.1f	72.5e	82.0c	91.0a	88.2b	80.2d
Dustlik	62.5f	73.0c	79.0a	81.1a	71.0d	68.0e
Uzbek-2	59.1d	61.0d	66.3b	69.3a	60.3d	64.2c

Means followed by identical letters horizontally are statistically alike at LSD ≤ 0.05

Table 2
Effect of different N rates on stem girth (cm)

Variety	N rate					
	N_0	N_{50}	N_{100}	N_{150}	N_{200}	N_{250}
Yuldus	1.00a	1.10a	1.24a	1.05a	1.10a	nd
Dustlik	0.92a	1.00a	1.10a	1.00a	1.25a	nd
Uzbek-2	1.00a	1.00a	1.10a	1.18a	1.16a	nd

Means followed by identical letters horizontally are statistically alike at LSD $\leq$ 0.05
nd – not determined

The number of leaves formed increased with a progressive increase in N rates (Table 3). All the varieties attained maximum leaf formation at the N_{100} level, ranging from a minimum of 46.7 to a maximum of 56.8 leaves per plant. There was a significant increase in plant density in all the varieties as the N rates increased, though it declined at the N_{250} level (Table 4). Optimum plant density (OPD) was obtained with N_{100} in two varieties, Yuldus and Uzbekskaya-2 with the latter attaining 79.9 thousand plants per hectare. The OPD for Dustlik was obtained at a higher (N_{200}) rate. Plants grown with the N_{250} rate were weak and appeared etiolated with thin stems and narrow leaves. The pod yield of all the varieties evaluated increased significantly with an increase in the N rate (Table 5). The optimum pod yield was obtained at N_{150} kg ha^{-1} in all varieties. A further increase in N rate up to 200 kg ha^{-1} had a negative effect on the number of pods obtained per plant. The grain yield was significantly increased by an increase in the N rate (Table 6). The highest yield was obtained for all the varieties at the N_{150} rate and ranged from 3.1 to 4.0 t ha^{-1}.

Table 3
Effect of different N rates on leaf formation (No./plant)

Variety	N rate					
	N_0	N_{50}	N_{100}	N_{150}	N_{200}	N_{250}
Yuldus	37.7b	37.3b	46.7a	36.7b	33.5c	33.5c
Dustlik	32.6c	36.8b	54.8a	35.8b	32.9c	32.7c
Uzbek-2	33.8c	38.9b	56.8a	39.0b	39.8b	34.9c

Means followed by identical letters horizontally are statistically alike at LSD $\leq$ 0.05

Table 4
Effect of different N rates on plant density (thousand/ha)

Variety	N rate					
	N_0	N_{50}	N_{100}	N_{150}	N_{200}	N_{250}
Yuldus	68.5c	72.6b	78.7a	76.9b	74.9d	65.8d
Dustlik	54.9e	60.5d	68.0c	70.0b	72.7a	68.6c
Uzbek-2	75.7b	77.0b	79.9a	80.0a	70.7c	68.5d

Means followed by identical letters horizontally are statistically alike at LSD $\leq$ 0.05

Table 5
Effect of different N rates on pod yield (No./plant)

Variety	N rate					
	N_0	N_{50}	N_{100}	N_{150}	N_{200}	N_{250}
Yuldus	65.1d	68.5c	98.4b	106.8a	60.0e	nd
Dustlik	68.1e	71.0d	97.9b	100.8a	87.4c	nd
Uzbek-2	105.7e	111.4d	124.3b	152.9a	115.1c	nd

Means followed by identical letters horizontally are statistically alike at LSD ≤ 0.05
nd – not determined.

Table 6
Effect of different N rates on grain yield (t/ha)

Variety	N rate					
	N_0	N_{50}	N_{100}	N_{150}	N_{200}	N_{250}
Yuldus	1.3c	1.4c	2.1ab	3.1a	1.1c	1.0c
Dustlik	1.3d	2.3c	2.9ab	3.9a	1.7d	1.1d
Uzbek-2	1.8c	2.7b	3.0ab	4.0a	2.1c	1.8c

Means followed by identical letters horizontally are statistically alike at LSD ≤ 0.05

Discussion

The results suggest that N rates exceeding 150 kg N ha^{-1} are antagonistic to soybean growth and development and thus result in poor pod and grain yields. Usually, the grain yield is used to measure the varietal response to N fertilization (Dashiell et al., 1987). However, Rao et al. (1981) reported yield increases of 66% and 108% for the varieties Bossier and TGm 294, respectively, in response to 180 kg N ha^{-1}. In Egypt, the recommended rate of N fertilizer application is also 180 kg N ha^{-1} in three equal dressings at irrigation, but farmers are reported to use higher rates, averaging 240 kg N ha^{-1}, in one major production zone, in the Menia governorate (Deuson et al., 1984).

Some scientists (Olrosh, 1964; Shukov, 1972; Kariagin, 1978) have opined that N fertilizer application under leguminous crops was unnecessary, because 40% to 70% of the N could be obtained through symbiotic N fixation. It is assumed that varieties with little or no increase in yield after N fertilization may have obtained an adequate supply of N from biological fixation due to nodulation with naturally occurring rhizobia. The N nutrition of leguminous crops, especially soybean, has been a very controversial research area among agronomists, and up till now there is no generally acceptable rate of N fertilizer application for soybean cultivation (Ogburia, 1987). From the present work and from the literature, it could be observed that the response of soybean to N nutrition is dependent on the variety and the N fertilizer rate and can be influenced to a large extent by both the agroecological environment, root nodulation ability and the production technology.

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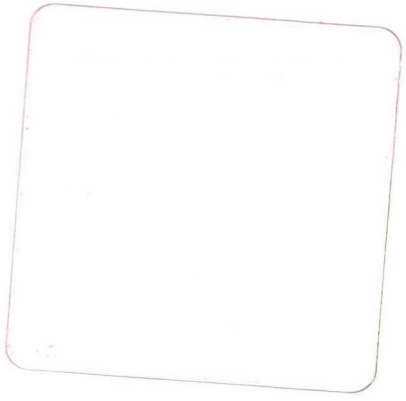

Book reviews

R. APPELS, R. MORRIS, B. S. GILL and C. E. MAY: Chromosome Biology. 1998. Kluwer Academic Publishers, Boston/Dordrecht/ London. 401 pp. ISBN 0-412-02601-5

Cytogenetics has made great progress during the last quarter of the 20th century mainly due to the application of novel molecular genetic and cellular biology tools and techniques. In this book comprehensive information is given about all aspects of cytogenetics. Chromosome Biology is a synthesis of modern viewpoints on chromosome structure, function and behaviour. The coverage is wide-ranging, from bacterial protosomes to wheat and human chromosomes. Chromosome Biology investigates the fascinating behaviour of chromosomes at mitosis and meiosis, and the structural and numerical chromosomal changes caused by genome manipulation and genetic engineering.

Chromosome Biology is divided into 8 parts. Each part has 3 chapters. Part 1 contains the introduction, a historical perspective of chromosome structure, function and behaviour, and discusses microscopes as basic tools for cytogenetic investigation. Part 2 describes chromosomes in the mitotic cell cycle, meiosis, synaptonemal complexes, the mechanism of crossing over, chromosome morphology, polytene, sex and B chromosomes. Part 3 is devoted to chromosome structures and rearrangements (deletions, duplications, inversions and reciprocal translocation). Great attention is given to the chromosome rearrangements in natural populations and to the relationship between chromosomal aberrations and cancer in humans. In part 4 chromosome polyploidy, aneuploidy and haploidy are discussed. Part 5 describes the location of genes using chromosome aberrations, and the transfer of alien genes by chromosome manipulations. The development and use of chromosome substitution and alien addition lines are given in detail. Part 6 illustrates the DNA organization and replication of protosomes and chromosomes. The formation of nucleosomes consisting of a core of four different histones with DNA wrapped around the outside is demonstrated. In part 7 a review is given of *in situ* hybridization and the molecular analysis of chromosomal landmarks. The main emphasis is placed on the genetic and molecular mapping of chromosomes. Part 8 deals with three important objectives: the analysis of cytoplasmic traits, genetic engineering, and organisms of importance to genetics and cytogenetics. The reasons for the genetic importance of 16 species including *Arabidopsis thaliana, Triticum aestivum* and *Homo sapiens*, are summarized.

Written by internationally recognized experts in cytogenetics, this book is the most authoritative work on the subject to date; it is suitable for use by professionals, undergraduate and post-graduate students of plant and animal sciences, genetics, molecular biology, plant breeding and related fields.

J. SUTKA

F. KOVÁCS, J. KOVÁCS and J. BANCZEROWSKI (eds): Ways and means for the environmentally sound development of agricultural production. Magyar Tudományos Akadémia, Agrártudományok Osztálya, Budapest, 1998. 185 pp. ISBN 963 508 072 7

This excellent volume is the latest in the strategic research series "Hungary at the millenium". It discusses problems arising in animal husbandry and crop production in various types of farm due to Hungary's application for EU membership.

The first chapter (authors: J. Dohy et al.) deals with the environmental protection aspects of animal husbandry in conformity with EU regulations. It is surprising to learn that the relationship between stock-farming and the environment is good in Hungary, as the number of pigs per km^2 is 3.5 times as great in Denmark and six times as great in Holland as it is in Hungary, while the ratio is even better for cattle and poultry. This means that environmental pollution problems can be solved more easily here compared to the average value (1.9) in the EU. One problem is that in large-scale stock-farms the land required for fodder production, grazing and manure placement is not always available.

The situation is not as favourable if we examine problems concerning stock-keeping technologies. Clear guidelines are provided in connection with manure treatment, smell emission, soil and groundwater pollution,

hygiene, waste disposal regulations and technology developments.

In addition to intensive animal husbandry, I. Steffler and I. Vinczeffy have elaborated an extensive stock-keeping programme satisfying environment and nature protection requirements. This was facilitated by the long years of research already carried out in this field by agriculturalists and botanists in Hungary. The present 1.283 million hectares of grasslands will increase due to the areas which will be removed from production after joining the EU. The stock numbers are low in comparison (only 20% of the grasslands are exploited). Various suggestions are made for the utilisation of these areas (meat production from horses, foals, game, – examined *ante mortem*, goats, alpacca, etc.). Naturally this will not replace traditional cattle and sheep farming.

The research team consisting of K. Molnár and F. Baska reports on a regional programme. A technique has been developed for using fish parasites as an indicator of the level of pollution of rivers in Eastern Hungary.

The first of the chapters on crop production, written by T. Németh, deals with the role of nutrient management. Two of the main dangers threatening soil reserves are discussed:

A) Soil degradation processes (water, wind erosion, soil acidification, salinisation, physical degradation, biological degradation, reduced buffering capacity, etc.).

B) Soil pollution.

A detailed analysis is given of N migration and its role in the pollution of subsoil water. The interesting statement is made that N losses from mineral fertilisers are lower than suggested and that the quantity of N entering the groundwater is no less after organic manuring than after mineral fertilisation. One failing of this excellent chapter is that it makes no mention of the reduction in the nutrients required per unit product which can be achieved through plant breeding.

The two chapters written by F. Kozár provide an excellent survey of the biodiversity of the insect fauna. The data indicate that the use of pesticides has not caused a decline in biodiversity. The effect of global warming is even more interesting. Insect species originating in Mediterranean areas can now be found in Hungary, while insects from the tropics have been observed in Italy.

There is obviously a need for studies on the relationship between insects and transgenic feed-plants, but no data on this topic are yet available in Hungary.

The chapter by P. Solymosi covers many aspects of the weed question. The dangers involved in the use of transgenic plant seeds are underlined by the results of long-term experiments reported by Beal et al. (covering the period 1879–1979) and Kozma (1912–1917), which indicated that the seeds of major weed species remain viable in the soil for an average of 30 years, and some for as long as 80–100 years.

Many data are listed on the development of herbicide resistance and on how damage can be reduced. Novel data are presented on the appearance of Mediterranean weed species. The increase in the CO_2 level has a more favourable effect on the development of C_3 weeds than on that of C_4 species. Little success has so far been achieved in experiments on microbial weed control.

An excellent survey on the Hungarian application of plant protection techniques is presented by J. Kovács, who informs us that 361 different active ingredients are used in Hungary in 732 different products. They can be grouped as follows: fungicides (including seed dressing agents) 17%, insecticides 22%, herbicides 45%, other 16%. The main crops to which they are applied are maize 31%, cereals 18%, oil-bearing plants 16.6%, vineyards and orchards 14%, sugarbeet 11%, other crops 9.7%.

The world data on the efficiency of protection are sobering: Europe 52.7%, USA 44.6%, Africa 33%. Yield losses range from 29.4–51.4%.

On a list of 30 sources of danger to human life, pesticides took 28th place, food colouring agents 26th and food preservatives 27th.

A very wide-ranging subject is covered by the chapter written by K. Kovács and E. Balázs, who discuss the role of biotechnology in environmental protection.

In addition to the familiar subject of biogas, the main aim is bioremediation (gas filters, denitrification systems, handling of solid waste, biowaste, biosensors). This is greatly promoted by transgenic plants resistant to pathogens and pests, thus reducing or eliminating the need for costly chemicals and the resulting pollution. They are also useful in reducing soil pollution.

The new, extremely varied tasks facing water management are summarised by L. Cselőtei, who also discusses the environmental protection aspects of growing vegetables under polythene and the tasks arising in the course of stock-farming.

The novel approach found in this book will make it of great interest to its readers.

A. BÁLINT

THE WORLD FOOD PRIZE

The World Food Prize Foundation requests nominations for the year 2000 World Food Prize, which recognizes outstanding individual achievement in improving the quality, quantity, or availability of food in the world. The Prize emphasizes the importance of a nutritious and sustainable food supply for all people and recognizes that improving the world's food supply for the long term depends on nurturing the quality of land, water, forests and other natural resources.

Nominees should be individiuals who have worked successfully toward this goal in any field involved in the world food supply, including food and agricultural science and technology, manufacturing, marketing, nutrition, economics, political leadership, social sciences and other related fields that have brought food to tables of a significant number of people across the world.

The laureate will receive $250,000 and a sculpture created by world-renowned designer Saul Bass. The award is based solely on individual achievement with no consideration of nationality, ethnicity, political persuasion, religion, sex, or age.

For a brochure detailing nomination procedures, contact The World Food Prize Office of the Secretariat, David Acker, College of Agriculture, Iowa State University, Ames, IA 50011-1050; tel. (515) 294-2883; fax (515) 294-9477; e-mail: bjelland@iastate.edu or http://www.wfpf.org

The deadline for submission or nominations for the year 2000 World Food Prize is December 31, 1999.

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INSTRUCTIONS TO AUTHORS

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1. **Manuscripts** must be written in standard grammatical English in three copies with one set of the original illustrations and should be submitted to Prof. József Sutka, Editor, ACTA AGRONOMICA, H-2462, MARTONVÁSÁR, P.O. Box 19, Hungary. Manuscripts should be typed double-spaced with wide margins (3–4 cm), on one side of A4 paper. Authors are encouraged to submit their manuscripts typed on an IBM-compatible computer, preferably using Microsoft Word. Always supply us with both the hard-copy (print out) version of your final text, illustrations and the floppy diskette. The original paper should not exceed 7 printed pages (approximately 16 typed pages including figures and tables). Before acceptance for publication the papers will be evaluated by reviewers.

2. Every original standard paper should be divided into the following **sections:** Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References. Manuscripts should be headed with the **title** of the paper, initial(s) of first name(s) and surname(s) of author(s), and the institute where the research was carried out. A **running title** not to exceed 50 letter spaces should be included on a separate sheet.

3. **Abstracts** are required for all the manuscripts. They should be limited to max. 200 words. Up to 8 **key words** should be added at the end of the abstract.

4. Genus and species **names, gene symbols** and **Latin words** are printed in *italics.* A single straight line should be drawn under such names if no italic script is available.

5. **Units** should conform to the International System of Units (SI).

6. **Figures** and **Tables** should be limited to the necessary minimum; tables, figures and figure captions should be submitted together with the manuscript on separate sheets. On the reverse side of these figures the names of the authors and the figure number should be written. Figures should be submitted in **camera-ready** form. Only original prints of photographic material can be printed. Coloured illustrations cannot be accepted.

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Examples:

Lazar, M. D., Schaeffer, G. W., Baenziger, P. S. (1984): Cultivar and cultivar × environment effects on the development of callus and polyhaploid plants from anther cultures of wheat. *Theor. Appl. Genet.,* **67,** 273–277.

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Ouyang, J. (1986): Induction of pollen plants in *Triticum aestivum.* In: Hu, M., Yang, M. (eds), *Haploids of higher plants* ***in vitro.*** Academic Press, Beijing, 26–41.

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9. One set of **proofs** will be provided, which should be returned to the Editor within 3 days of receipt. Alterations in the text and especially in the illustrations should be avoided.

10. The corresponding author will be supplied with one free copy of the relevant issue.

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CONTENTS

ORIGINAL PAPERS

Acta Agronomica Hungarica, 47(4), pp. 337–345 (1999)

RADIOSENSITIVITY OF GRAPEVINES. II. EMPIRICAL MODELLING OF THE NET PHOTOSYNTHESIS AND PHOTORESPIRATION OF GRAPEVINES AS AFFECTED BY X-RAY IRRADIATION*

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Empirical models were utilized in depicting the net photosynthesis of grapevine clones resistant and sensitive to X-ray irradiation. It was revealed that the radiosensitivity of grapevine clones could involve an elevated level of net photosynthesis. This phenomenon could be coupled to lower photorespiration. An X-ray-induced mechanism, opposite to that supposedly taking place in the dissipation of the excess of light, was hypothesized.

Key words: ionizing irradiation, radioresistance, *Vitis vinifera*, net photosynthesis, photorespiration, models

Abbreviations: P_{nA}, net photosynthesis measured in air; P_{nN}, net photosynthesis determined in nitrogen gas; R_{ph}, photorespiration

Introduction

Grapevines, like other plants, harvest light energy to drive photosynthesis and support autotrophic growth, which is primarily dependent on the rootstock genotype and plant age (Düring, 1994). Photorespiration is the light-dependent evolution of CO_2 in C_3 plants, which derives from glycine oxidation and causes a reduction in the rate of photosynthetic CO_2 assimilation (Zelitch, 1979). It is well documented in the literature that plants have developed various metabolic processes to protect the photosynthetic apparatus from damage by an excess of light. The detrimental effects exerted on photosynthesis are increased by extremes of various environmental stresses (Ludlow and Powles, 1988). It has been suggested that photoinhibition and photorespiration are able to protect plants from excess light energy (Öquist et al., 1992). In this process antioxidants, zeaxanthin levels and D_1 protein turnover have also been implicated (Iacono and Sommer, 1996).

In the present experiment, the Chardonnay Clone type, found to be the most resistant in earlier empirical modelling, and the most sensitive genotype, Cabernet Sauvignon E. 153, were involved. The goal was to distinguish between radiosensitive and -resistant grapevines in relation to the parameters P_{nA}, P_{nN} and R_{ph}.

*Paper presented at the IMACS/IFAC Second International Symposium on Mathematical Modelling and Simulation in Agricultural and Bio-industries, May 7–9, 1997 Budapest, Hungary.

0238–0161/99/$ 5.00

Materials and methods

Plants, growing conditions and irradiation

Single-bud cuttings of clones of Cabernet Sauvignon E. 153 and of the Chardonnay clone type were involved in the experiments. Liliput 140 X-ray apparatus (Medicor, Hungary) was used for the low linear energy transfer irradiation considered to be more appropriate for plant radiobiological purposes (Kőrösi, 1991). The treatment was performed in the Central Laboratory of the University of Agricultural Sciences, Gödöllő. One-bud cuttings were irradiated with 400 Gy [120 kV, 4.5 mA]. This manipulation represented the irradiated state. After irradiation, the cuttings were put in pots containing an appropriately moistened mixture of peat and perlite (3:1, v/v). The shoots were allowed to grow in a chamber at a temperature of 22 ± 2°C, relative humidity 60–80%, under a low light intensity of 200 μE m^{-2} s^{-1} photosynthetic photon flux density (PPFD), till the control plants reached the 4–5-leaf development stage.

Measurement of net photosynthesis (P_{nA} and P_{nN}), calculation of photorespiration (R_{ph})

Before measuring P_{nA} and P_{nN}, the shoots were light-adapted for 30 min. to 1000 μE m^{-2} s^{-1} PPFD. This PPFD was assumed to be below the level of light saturation for grapevines (Düring, 1988) and represented high flux density (Chaumont et al., 1995). The P_{nA} and P_{nN} measurements were performed on light-adapted leaves, in a closed measuring chamber, in relation to the external CO_2 concentration gradients and defined as μM CO_2 mol^{-1}. In order to estimate the CO_2 absorption an infrared gas analysing method (IRGA) was employed. The temperature in the gauging chamber was adjusted to 25°C with a light intensity of 1000 μE m^{-2} s^{-1}. Measurements were carried out in both air (P_{nA}) and nitrogen (P_{nN}). The CO_2 uptake was related to the dry weight of the shoots {μM CO_2 g $(DW)^{-1}s^{-1}$}.

Mathematical model

To model the P_{nA} and P_{nN} of the radiosensitive genotype Cabernet Sauvignon E. 153 and the radioresistant Chardonnay clone type a simple linear probabilistic model was set up:

$$\hat{E}(y) = \hat{\beta}_0 + \hat{\beta}_1 x \qquad (1)$$

where $\hat{E}(y)$ = P_{nA} or P_{nN} {μM CO_2. g $(DW)^{-1}.s^{-1}$}, $\hat{\beta}_0$ = y intercept, $\hat{\beta}_1$= CO_2 concentration {μM $CO_2.mol^{-1}$} (Mendenhall, 1987; Walpole and Myers, 1989; Harsbarger and Reynolds, 1989). Both confidence and prediction intervals were given at the 95% level for the parameters. The values of R_{ph} were calculated using the estimating equation (1) for P_{nA} and P_{nN}.

$$R_{ph} = P_{nN} - P_{nA} \qquad (2)$$

To characterize the effect of irradiation on P_{nA} and P_{nN} a multiple regression model proved to be suitable:

$$\hat{E}(y) = \hat{\beta}_0 + \hat{\beta}_1 x_1 + \hat{\beta}_2 x_2 + \hat{\beta}_3 x_1 x_2, \qquad (3)$$

where $\hat{\beta}$ = constant, x_1 = μM $CO_2.mol^{-1}$, x_2 = a qualitative (indicator) variable with a value of 1 if irradiated with 400 Gy and 0 if non-irradiated.

In order to determine whether a change in P_{nA} or P_{nN}, corresponding to a change in CO_2 concentration, depended upon the irradiation state (non-irradiated or irradiated with 400 Gy), the interaction term x_1x_2 was introduced into the model. For the mathematical evaluations the Statgraf 4.0 computer program was used.

Results

Net photosynthesis

In Figs 1 and 2 the net photosynthesis of the grapevines ascertained in air and nitrogen gas and model values are shown with their estimation, confidence and significant levels.

When comparing the varieties (clones) in the non-irradiated state (Fig. 1A and C; Fig. 2A and C) neither $\hat{\beta}_0$ nor $\hat{\beta}_1$ differed from one another. This indicates that clones with highly different responses to X-ray irradiation did not exhibit differences in their net photosynthesis, determined both in air and nitrogen.

A very different picture emerged when the effect of X-rays on the net photosynthesis was considered. As a result of irradiation $\hat{\beta}_1$ was significantly higher in the model for the extremely sensitive Cabernet Sauvignon E. 153. Consequently, the net photosynthesis and the estimated value at a concentration of x_p = 300 μM CO_2 mol^{-1} were augmented by irradiation at 400 Gy (Table 1). This effect was more pronounced in air (+121%) than in N gas (+34%). The regression coefficient of P_{nA} for Cabernet Sauvignon E. 153 in air was more than twice as high (0.18 *vs.* 0.41) as that for unirradiated shoots (Fig. 1A and B). However, in the resistant Chardonnay clone type the net photosynthetic model parameters did not differ significantly under the influence of X-ray radiation. At x_p = 300 μM CO_2 mol^{-1} there tended to be a slight decrease in the net photosynthesis (Table 1).

Table 1
Modeled net photosynthesis as affected by irradiation at x_p= 300 μM CO_2 mol^{-1}

Treatment	Non-irradiated {μM CO_2 g $(DW)^{-1}s^{-1}$}				Irradiated {μM CO_2 g $(DW)^{-1}s^{-1}$}				As a % of the control
	n	Estimated value	95% confidence interval		n	Estimated value	95% confidence interval		
			LCL	UCL			LCL	UCL	
CS-E. 153*	74	41.24	35.77	46.71	67	91.15	80.00	102.30	221.02
CS-E. 153**	47	63.22	53.79	72.65	55	84.85	77.66	91.74	134.21
C*	71	47.43	33.11	61.75	69	44.98	39.94	50.02	94.83
C**	45	75.85	67.53	84.17	53	63.94	53.23	74.65	84.28

LCL = Lower Confidence Limit; UCL = Upper Confidence Limit; CS-E. 153 = Cabernet Sauvignon E. 153; C = Chardonnay clone type; * = measurement in air, ** = measurement in nitrogen gas

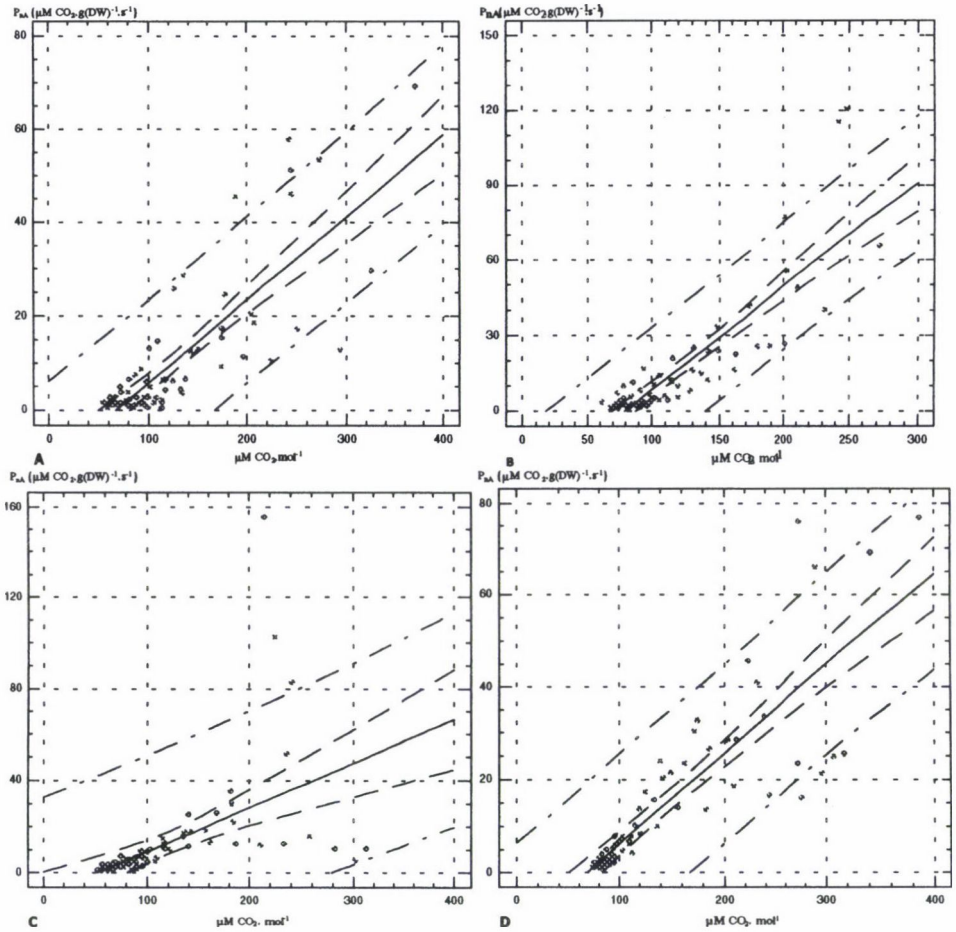

Fig. 1. Net photosynthesis of Cabernet Sauvignon E. 153 shoots developed from (A) non-irradiated (n=74) and (B) irradiated buds (n=67), and of Chardonnay shoots developed from (C) non-irradiated (n=71) and (D) irradiated buds (n=69). Solid line: estimation of expected net photosynthesis [μM CO_2 g(DW)$^{-1}$ s^{-1}]; dashed lines: 95% confidence interval of the expected values; dashed-dot lines: 95% prediction interval. X axis: CO_2 concentration of the air [μM CO_2 mol^{-1}]. Measurements were performed in air

Dependence of net photosynthesis on the state of irradiation

In order to ascertain if the net photosynthesis of sensitive and resistant grapevine varieties was dependent on irradiation the interaction term between the CO_2 concentration (μM CO_2 mol^{-1}) and irradiation (400 Gy) was analysed. As can be seen from the model (see p values, Table 2) the interaction term (β_3) for Cabernet Sauvignon E. 153, which was sensitive to X-ray irradiation, was highly significant, whereas no change was observed for the resistant Chardonnay clone type. If β_3 was significant in the multiple regression model, the reliance of net photosynthesis in Cabernet Sauvignon E. 153 on μM CO_2 mol^{-1} concentration depended on the irradiation state. The resulting models are demonstrated in Fig. 3.

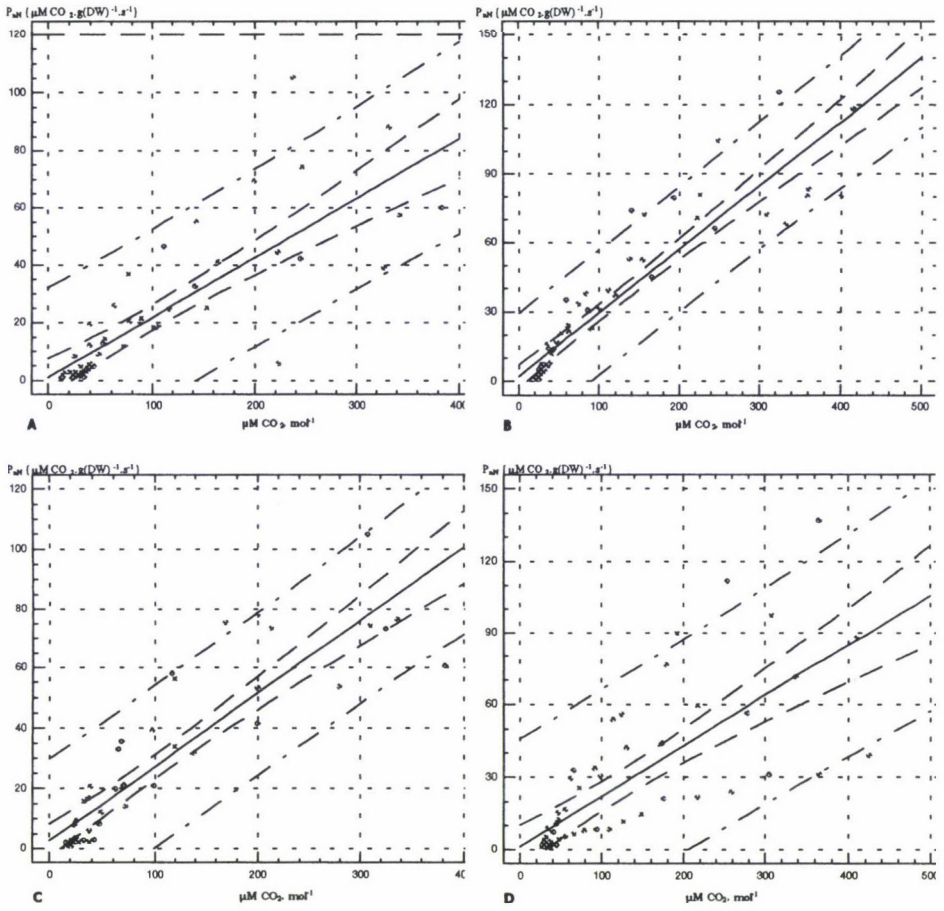

Fig. 2. Net photosynthesis of Cabernet Sauvignon E. 153 shoots developed from (A) non-irradiated (n=47) and (B) irradiated buds (n=55) and of Chardonnay shoots developed from (C) non-irradiated (n=45) and (D) irradiated buds (n=53). Solid line: estimation of expected net photosynthesis [µM CO_2 g $(DW)^{-1}$ s^{-1}]; dashed lines: 95% confidence interval of the expected values; dashed-dot lines: 95% prediction interval; X axis: CO_2 concentration of the nitrogen gas [µM CO_2 mol^{-1}]. These measurements were made in nitrogen gas to determine the photorespiration

Regulation of net photosynthetic activity of grapevines by photorespiration under the influence of X-ray irradiation

As is well known, photorespiration is a characteristic biochemical feature of C_3 plants. This process is also implicated in protecting the photosynthetic apparatus from excess light. As a result of a 400 Gy dose of irradiation a detrimental effect was noticed in the sensitive genotype Cabernet Sauvignon E. 153 and the whole system appeared to have ceased functioning at a concentration of > ~280 µM CO_2.mol^{-1} (Fig. 4A). At the same time for the Chardonnay clone type, presumed to be resistant to X-ray irradiation, a slight adaptation effect may have occurred and the enzymatic systems and pathways seem to have remained functional in the experimental concentration range of 0 + 500 µM CO_2 mol^{-1}.

Table 2
Interaction term of the multiple regression model

Treatment	n	R^2	$\hat{\beta}_3$			
			Estimated value	p value	95 % confidence interval	
					LCL	UCL
CS-E. 153*	141	0.72	0.23	0.00	0.17	0.29
CS-E. 153**	102	0.79	0.07	0.01	0.01	0.13
C*	140	0.41	0.00	0.91	–0.08	0.93
C**	98	0.64	–0.04	0.30	–0.11	0.03

LCL = Lower Confidence Limit; UCL = Upper Confidence Limit; CS-E. 153 = Cabernet Sauvignon E. 153; C = Chardonnay clone type; * = measurement in air, ** = measurement in nitrogen gas

Discussion

The genetic background plays a key role in differences in the net photosynthesis of grapevines (Zhu et al., 1994; Düring, 1994). Besides genetic factors many other determinants, such as leaf position, light level, leaf age, temperature, plant training and mineral nutrition affect the carbohydrate assimilation in grapes (Koblet and Candolfi, 1995; Bica and Novello, 1995; Ferrini et al., 1995; Schubert et al., 1995). Diurnal and seasonal physiological changes also influence the CO_2 assimilation rate (Hunter et al., 1994).

To the best of our knowledge, the connection between the net photosynthesis and photorespiration of grapevines and radiosensitivity has not yet been studied. Hence, as a continuation of earlier studies on radiosensitivity (Hajdu et al., 1994; 1995) photosynthesis (P_{nA} and P_{nN}), the main energy-producing process of plants, and photorespiration (R_{ph}) were investigated.

In the non-irradiated state there was no significant difference in the net photosynthesis either when measured in air (P_{nA}) or in nitrogen gas (P_{nN}) (Figs 1 and 2). However, under the influence of X-ray irradiation there was a significant augmentation in P_{nA} and P_{nN} for Cabernet Sauvignon E. 153, which was sensitive to X-ray irradiation (Table 1). At the same time there was no change for the resistant clone type Chardonnay. By applying a multiple regression model it was proved that the dependence of net photosynthesis on the CO_2 concentration was a function of the irradiation state in the sensitive genotype Cabernet Sauvignon E. 153 (Table 2). This relationship was not established for the resistant Chardonnay clone type.

Decreased photorespiration may play a key role in the maintenance of excessively high net photosynthesis in the sensitive genotype Cabernet Sauvignon E. 153, though the fact that photorespiration ceased to function above 280 μM CO_2 mol^{-1} may indicate injury to the photorespiration system (Fig. 4A). The latter did not occur to such an extent for the resistant Chardonnay clone type (Fig. 4B). Generally, in the irradiated state, the higher the external CO_2, the greater the slowdown in photorespiration. On the other hand, less C loss occurred due to

photorespiration. It is postulated that a low intensity maintenance of photorespiration might well be one of the properties characterizing the biochemical pathways of radiotolerance in the Chardonnay clone type. This postulated, X-ray-induced mechanism seems to act in an opposite direction to that suggested for the protection of the photosynthetic apparatus from excess light *via* an increase in activity, thereby dissipating the excess energy, especially under stressed conditions (Düring, 1988; Öquist et al., 1992; Ögren and Rosenquist 1992; Iacono and Sommer, 1996).

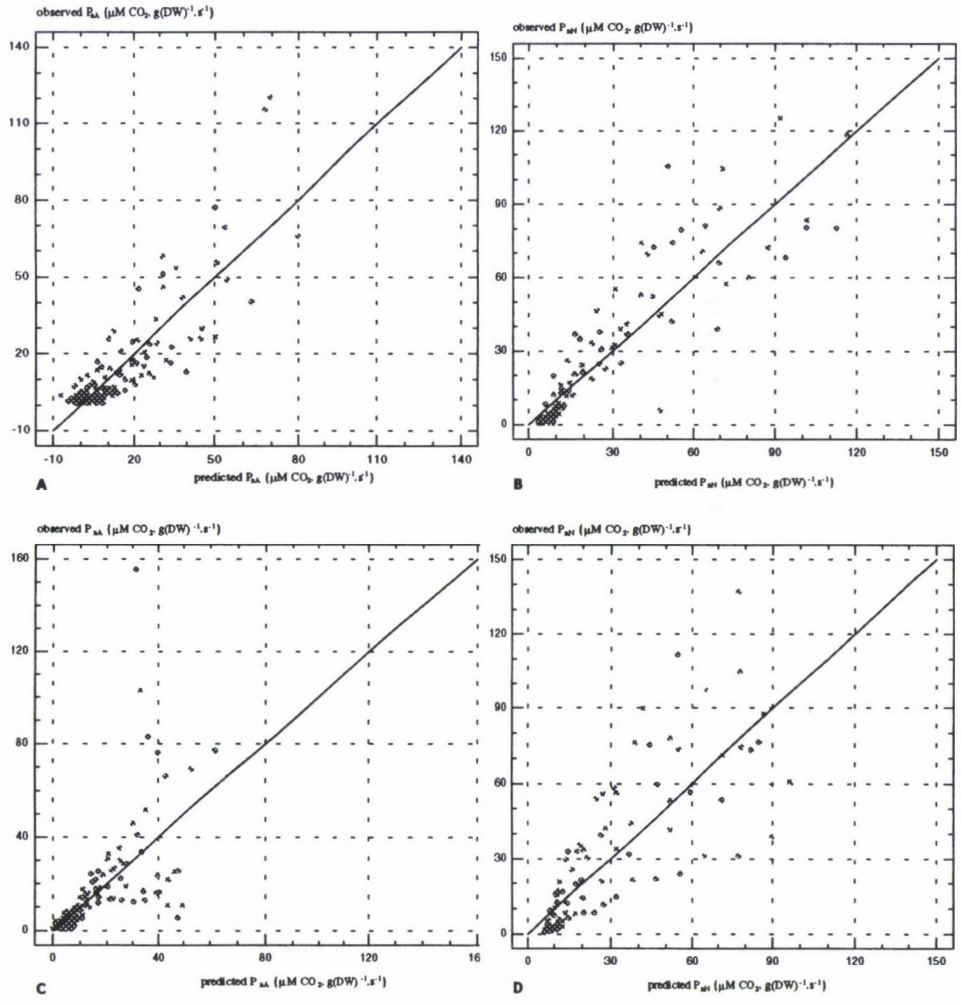

Fig. 3. Multilinear model of net photosynthesis as affected by X-ray irradiation. (A) and (B): Cabernet Sauvignon E. 153 shoots, measured in air (n=141) and nitrogen gas (n=102), respectively; (C) and (D): Chardonnay, measured in air (n=140) and nitrogen gas (n=98), respectively.

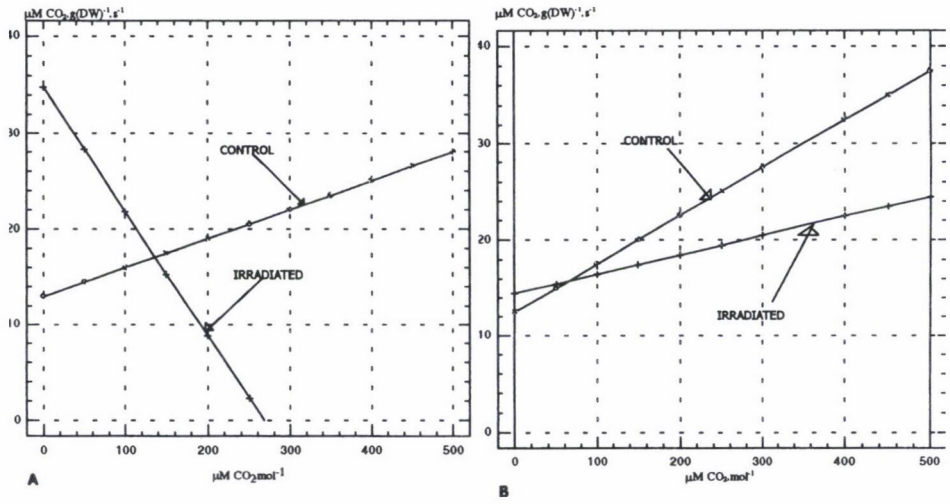

Fig. 4. Calculated photorespiration of Cabernet (A) and Chardonnay (B) using estimating equations

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TESTING OF DROUGHT TOLERANCE IN WHEAT VARIETIES ON THE BASIS OF PHOTOSYNTHETIC AND O_2 SCAVENGING PERFORMANCE

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The goal of this study was to reveal the relationship between the stress tolerance capacity and easily recorded physiological responses of wheat varieties with known drought tolerance in the field. The peroxidase activity was lower in varieties with outstanding drought tolerance than in varieties less resistant to water deficiency. The superoxide dismutase activity was similar in both the treated and control plants of all the varieties examined, being only slightly lower than average in treated samples of the most drought-tolerant variety, Fatima. The fresh and dry masses of the roots and shoots of the varieties exhibited a correlation with their drought tolerance: the reduction in fresh mass was less intense in varieties with drought tolerance, while the dry mass of the roots and shoots increased in direct proportion to the drought tolerance capacity. The CO_2 fixation dropped sharply as a function of the percentage water deficit in the roots, while the total chlorophyll content of the plants exhibited smaller but significant changes. As the result of a long period of moderate water deficiency changes were observed in the amounts and ratios of the photosystem I, photosystem II and light-harvesting II complexes, reflecting acclimative alterations in the drought-tolerant varieties and degradative changes in more sensitive ones. The four wheat varieties examined formed two very distinct groups as regards drought tolerance. Two of the Martonvásár varieties carrying the 1B/1R wheat/rye translocation (Mv 17 and Fatima) were more tolerant of long periods of moderate water deficiency, while the third Martonvásár variety (Magma) and the UK variety Hereward proved to be more sensitive to water stress. The correlation revealed between the drought tolerance of the wheat varieties and physiological parameters (particularly peroxidase activity and changes in the ratios of pigment-protein complexes) makes it possible to predict the drought tolerance of the varieties by testing seedlings in the laboratory.

Key words: drought tolerance, wheat, peroxidase, superoxide dismutase, CO_2 fixation, pigment-protein complexes

Abbreviations: LHC, light harvesting complex; PER, peroxidase; PS, photosystem; SOD, superoxide dismutase

Introduction

Both long-term and temporary drought cause considerable yield losses in cereal production worldwide. Under Hungarian climatic conditions temporary drought or moderate water deficiency is a frequent occurrence during the vegetation period of wheat. The breeding and cultivation of drought-tolerant varieties is one of the main aims of wheat breeding.

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The traditional methods used for the evaluation of drought-tolerant genotypes and varieties developed by means of classical breeding methods or biotechnological techniques are both expensive and time-consuming. Various methods are also available for the laboratory evaluation of the drought tolerance of woody and herbaceous plants. Enzyme reactions which play a role in the elimination of the harmful reactive oxygen species (ROS) formed under stress conditions are one group of physiological processes linked with drought tolerance. The superoxide dismutases (SOD, E.C. 1.15.1.1) react directly with superoxides and neutralise them, producing H_2O_2, while the heterogeneous group of peroxidases (PER, E.C.1.11.1.7) transfer the peroxo group into various substrates. Various isoforms of these enzymes function in different compartments. They are induced by stress effects and continue to exhibit increased activity during the regeneration following stress treatment (Mittler and Zilinskas, 1994). Their activity increase is based partly on post-translational regulation and partly on *de novo* synthesis (Gupta et al., 1993). High PER and SOD activity is indicative of the existence of oxidative stress, i.e. a given ROS level, and is thus inversely proportional to the drought tolerance ability (Loggini et al., 1999).

Drought stress is one of the most important environmental factors inhibiting photosynthesis (Bradford and Hsiao, 1982). PSII was shown to be particularly sensitive to severe water deficiency (Masojidek et al., 1991; van Rensburg and Krüger, 1993). The lowering of its activity could be connected either with the down-regulation of PSII electron transport or with direct damage to the PSII reaction centres related to the increased rate of degradation of D_1 protein (He et al., 1995; Giardi et al., 1996). Moderate water deficiency only had a slight effect on the maximum PSII efficiency (Genty et al., 1987; Stuhlfauth et al., 1990; Cornic and Briantais, 1991). CO_2 fixation was influenced not only by the changed activity of the photosynthetic apparatus, but by the state of the stomata depending on the drought stress (Gollan et al., 1986).

The studies described below were carried out on four wheat varieties (Fatima, Mv 17, Magma, Hereward) for which drought tolerance data were available from field experiments. Three of these winter wheat varieties were bred in Martonvásár (Fatima, Mv 17, Magma) and bear the 1B/1R wheat/rye translocation (Lángné et al., 1996). The presence of a chromosome consisting of the long arm of wheat 1B and the short arm of rye 1R is associated with a number of favourable agronomic properties, such as resistance to fungal diseases, greater yield, etc. (Bedő et al., 1993). Due to the differing genetic backgrounds, however, there are considerable differences between the varieties, for example in yield. A connection between the 1B/1R translocation and drought tolerance has not yet been proved.

The results of laboratory investigations on the drought tolerance of the varieties examined agreed on the whole with those of field studies. The data proved that the measurement of PER activity could be used as a rapid test of drought tolerance, while the determination of the amounts and ratios of pigment-protein complexes could reveal whether the given variety was capable of adapting to or surviving stress conditions.

Materials and methods

Plant materials

Seeds of wheat (Fatima, Mv 17, Magma, Hereward) were sterilised in 3% H_2O_2 for 15 min, continuously rinsed for 1 h under running tap water and then germinated on wet filter paper at 25°C in the dark in a controlled growth chamber. After 2 days the seedlings were transferred to hydroponics (1/4 strength Hoagland nutrient solution) in a thermostated (25°C) growing room at 16 W m^{-2} irradiation intensity with a 12/12 h photoperiod.

Stress treatment

Plants were stressed from the age of 5 days for daily extending periods of time (20, 40, 60, 80, 100, 120 min) by taking their roots out of the medium, towelling them with filter paper and leaving them dry for the given time. The extraction of SOD and PER was carried out daily just after the stress treatment. Growth parameters, photosynthetic performance and structural changes in the thylakoids were examined after prolonged stress by harvesting the seedlings on the 10th day of cultivation.

Preparation of crude extracts

To obtain crude extracts of the enzymes, 0.5 g leaf material was extracted with 300 µl buffer [0.5 M phosphate buffer, 0.2 mM EDTA, 0.005% (v/v) Triton X-100 and 0.2% (w/v) PVP-25] (Karataglis et al., 1991). The homogenates were centrifuged at 10 000 g for 15 min. The supernatant was stored in liquid nitrogen until required for electrophoresis.

Native PAGE and activity staining

PER isoenzymes were separated with a Pharmacia™ Phast System apparatus on Phast gels (4–15% AA) using electrode buffer strips (LiOH 26 mM, pH 8.0). All electrophoretic separations were performed at 10°C. After migration the gel was soaked in the staining solution [5 mg o-dianisidine dissolved in 0.5 ml N,N-dimethylformamide, 10 ml sodium acetate buffer (0.1 M, pH 4.4), 10 µl 2 M $CaCl_2$ solution and 20 µl 30% H_2O_2] for 10 min (Miller et al., 1990). Areas of PER activity showed up as light brown bands. After the bands had developed the gels were scanned, then stored in sodium acetate buffer (pH 4.4). Only the active area of the scanned gel images is shown here.

SOD was analysed on gradient PAGE and visualised by negative staining, using the method of Beauchamp and Fridovich (1971) with minor modifications. PAGE was run on 6–12% gradient gels (Tris-phosphate, pH 6.5) in a Pharmacia Phast System apparatus with a LiOH–H_3BO_3 buffer strip at 16°C.

Each sample contained 30 µg protein. Protein content was measured according to Bradford (1976) and the crude extracts were diluted with extraction buffer when necessary.

Determination of photosynthetic parameters

The chlorophyll content of the leaves was determined in 80% acetone, using the equations of Porra et al. (1989). Chlorophyll-proteins were separated by Deriphat PAGE using mainly glucosidic detergents (dodecyl sucrose : nonyl glucoside : lithium dodecyl sulphate = 4.5:4.5:1) for the solubilisation of the thylakoids (Sárvári and Nyitrai, 1994). They were identified from the polypeptide patterns obtained on 10–18% gradient gels according to Laemmli (1970). The quantity of the pigmented bands was calculated from densitograms measured at 671 nm. The amount of a given complex was obtained by summing up the densitogram bands corresponding to this complex, after which the chlorophyll content of the seedling was shared among the complexes according to their relative proportions. The CO_2 fixation activity was determined according to Láng et al. (1985), and the incorporated ^{14}C was measured with a liquid scintillation apparatus (Beckmann LS 5000TD).

Statistical analysis

The values were the means of 5 measurements taken from 3 separate experiments.

Determination of percentage water deficit (WD)

WD was calculated on the basis of the fresh and dry mass of the control and treated plants, where the dry mass was measured after drying the plants at 80°C to constant mass. The value of the actual water content was given by the difference between the fresh and dry mass. The water deficit in stressed plants was calculated as the difference between the 'normal' water content and the actual water content. The 'normal' water content of stressed plants (an extrapolation of their water content to the 0 stress treatment) was calculated using the following equation:

$$\text{'normal' water content} = 100S_{dm}/C_{dm}\% - S_{dm}$$

where S_{dm} is the dry mass of the stressed plants and $C_{dm}\%$ is the percentage dry mass of the control plants ($100\ S_{dm}/C_{dm}\%$ is the fresh mass of stressed plants extrapolated to the 0 stress treatment). The percentage water deficit is the ratio of the water deficit to the 'normal' water content, calculated as a %.

Results and discussion

Among the four wheat varieties the PER activity was the lowest in Fatima, indicative of a low level of oxidative stress within the cells and a low H_2O_2 level (Fig. 1). While the activity exhibited in stress-treated Mv 17 and Fatima was close to that of the control, the PER activity in the two less stress-tolerant varieties (Hereward and Magma) increased substantially as the result of the treatment compared with the control. Similar results were obtained by Loggini et al. (1999) when the glutathione reductase (GR) and hydrogen peroxide-glutathione peroxidase activities of Italian wheat varieties with various degrees of drought tolerance were compared. GR is the key enzyme in the Haliwell-Asada cycle and, like the peroxidases and superoxide dismutases, plays a role in the elimination of ROS. As the result of drought stress the GR activity in the less drought-tolerant variety increased substantially, while that of the drought-tolerant variety remained at a low level.

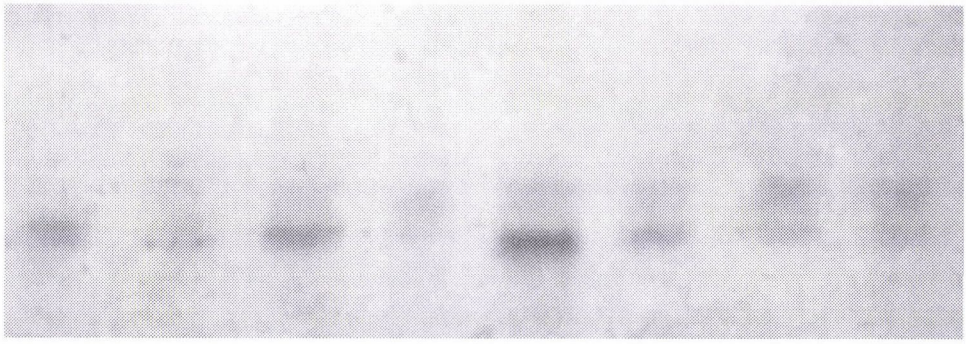

Fig. 1. Changes in PER patterns developed using native PAGE and activity staining after moderate stress treatment (40 min). From left to right: Fatima treated, Fatima control, Magma, Magma control, Hereward, Hereward control, Mv 17, Mv 17 control

The SOD activity was similar in all the samples; a slightly lower value than the average was only obtained for Fatima (Fig. 2). Based on the activity of enzymes induced by oxidative stress, two Martonvásár lines bearing the 1B/1R translocation (Fatima and Mv 17) formed a more drought-tolerant group, while the other Martonvásár variety, Magma, and the UK variety Hereward responded more sensitively to the application of moderate drought stress. Since Magma also carries the 1B/1R translocation (Lángné et al., 1996) the link between the 1B/1R translocation and drought tolerance is ambiguous. Although the varieties are similar in bearing the 1B/1R translocation, there are considerable differences in their genetic backgrounds, as revealed by Bedő et al. (1993) in tests on yield, breadmaking quality and resistance.

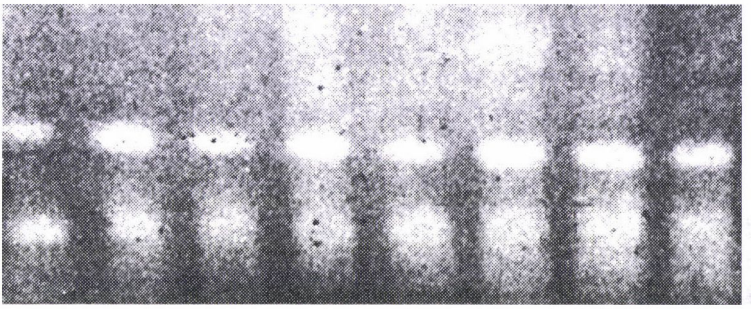

Fig. 2. Changes in SOD patterns developed using native PAGE and negative activity staining after moderate stress treatment (40 min). From left to right: Fatima treated, Fatima control, Magma, Magma control, Hereward, Hereward control, Mv 17, Mv 17 control

A comparison of the fresh mass data of the shoots and roots suggested the same grouping as above: the greatest reduction in fresh mass was observed for the varieties Magma and Hereward (Fig. 3A, B). The quantification of the changes (the slope of the curves presented in Fig. 3) is given in Table 1. The more pronounced tendencies observed for roots were due to the fact that the drought stress was applied through the roots, which naturally caused greater changes in the water content of the root tissues than in those of the shoots. The dry mass values of the shoots rose as the result of the stress treatment; the increase was greatest for Fatima and Mv 17, while Hereward was the only variety tested which had a lower dry shoot mass than the control (Fig. 3C). The higher dry mass of the shoots in the more drought-tolerant varieties might be connected with the greater accumulation of osmotically active substances. However, the dry mass of the roots did not change (Mv 17 and Fatima) or even decreased (Magma and Hereward) (Fig. 3D), again indicating the more severe stress exposure of the roots.

The CO_2 fixation capacity, indicative of the whole photosynthetic activity, seemed to be the most sensitive parameter of the drought-stressed plants (Fig. 3E). This is no doubt connected with the partial closure of the stomata as the consequence of the repeated stress treatment (Gollan et al., 1986). The chlorophyll content, which is a general indicator of structural and related

functional changes in the thylakoids, declined to various degrees in the different varieties, not only in the whole plant (Fig. 3F), but also per g fresh mass (not shown). In both cases, the greatest reduction was observed for Hereward, which also showed the greatest changes in the other parameters.

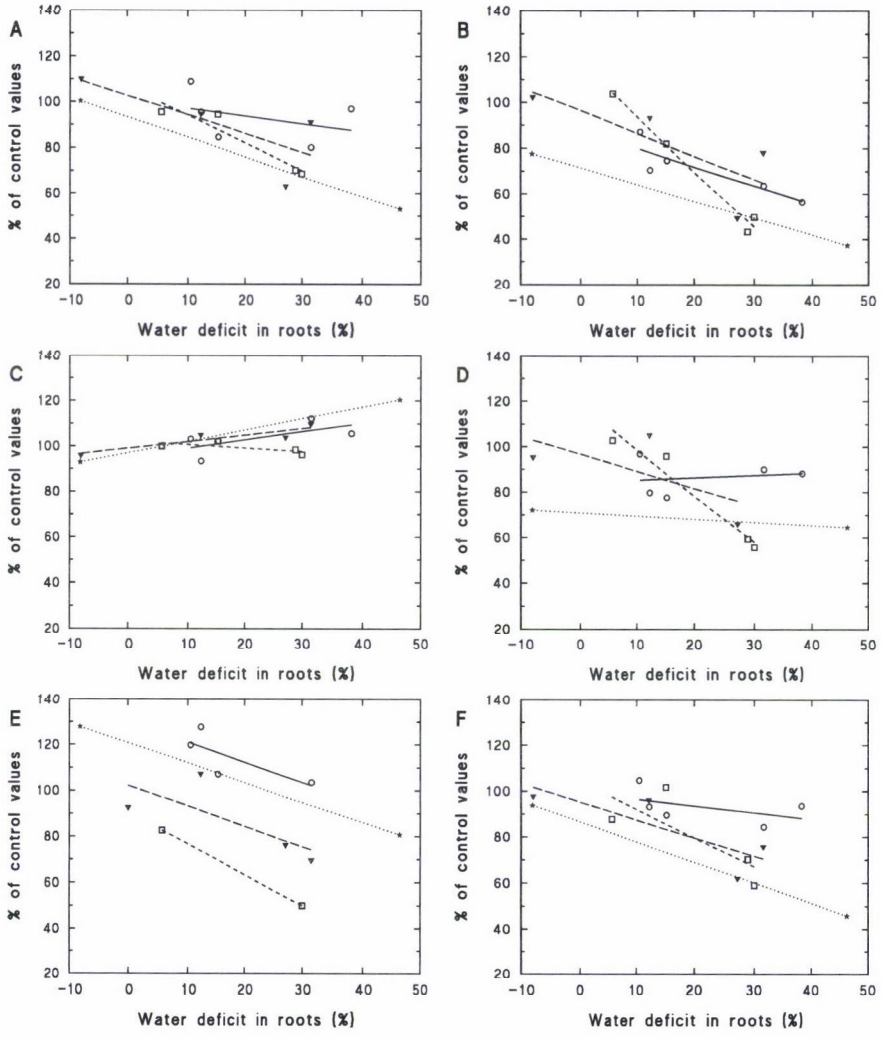

Fig. 3. Changes in parameters expressed as a percentage of control values and plotted as a function of the percentage water deficit in the roots. Fresh mass of shoots (A) and roots (B), dry mass of shoots (C) and roots (D), CO_2 fixation (E) and chlorophyll content (F) (·····*Fatima, ----•Magma, ----□ Hereward, ——o MV17 seedlings.)

Table 1
Extent and tendency of changes in the given parameters under drought stress
(slope of the curves presented in Fig. 3)

Varieties	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight	CO_2 fixation	Chl content
Hereward	–1.206	–2.403	–0.152	–2.025	–1.336	–1.252
MV 17	–0.335	–0.820	0.365	0.209	–0.874	–0.297
Magma	–0.821	–1.002	0.290	–0.758	–0.889	–0.789
Fatima	–0.869	–0.739	0.506	–0.138	–0.866	–0.882

The structural changes in the photosynthetic apparatus can be described more precisely if changes in the ratios of the pigment-protein complexes are considered, from which conclusions can be drawn on probable changes in the light-harvesting properties and photochemical activities of the system (Fig. 4). In Mv 17, which proved to be the most drought-tolerant on the basis of the other parameters tested, the PSII accumulation was slightly inhibited. Data from the literature also indicate that PSII is the complex whose activity and quantity respond the most intensively to the various effects (Chow et al., 1990). The decreased amount of PSII might be caused either by its decreased synthesis or by the increased degradation rate, under stress conditions, of the D_1 protein, the lack of which destabilises the PSII centres (He et al., 1995; Giardi et al., 1996).

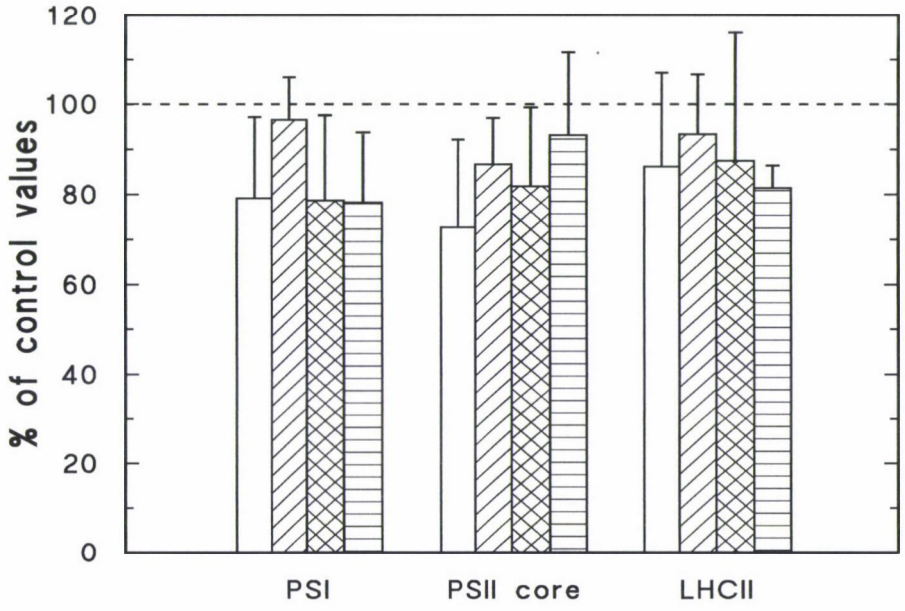

Fig. 4. Changes in the absolute quantities of pigment-protein complexes in whole seedlings as the result of a longer period of moderate water stress. Hereward (empty), Mv 17 (/), Magma (x) and Fatima (-)

The results obtained for Fatima, where PSII proved to be relatively more stable, suggest a slightly more intense stress response under photoinhibitory conditions (Sárvári et al., unpublished results). Photoinhibition may occur even at low light intensity in stressed plants which are less active photosynthetically (Janda et al., 1994; Horton et al., 1996). The greater stability of PSII in this case may be caused by the formation of non-degrading, dissipative centres which convert the surplus light energy into heat (Aro et al., 1993). As the result of high light intensity the acclimative proteolysis of LHCII was observed (Yang et al., 1998). In varieties shown by other analyses to be more sensitive (Magma and Hereward), complexes involving reaction centres (PSI and PSII) were found to be unstable even after moderate drought stress, while LHCII showed greater stability. This suggests the occurrence of degradation processes similar to those observed during aging (Humbeck et al., 1996). The particular instability of PSI may be induced by the insufficient activity of protective mechanisms against ROS (Sonoike, 1996).

It can be seen from the above results that of the four wheat varieties examined, the changes in the amount of pigment-protein complexes in Mv 17 and Fatima exhibited responses of the acclimative type after treatment, so both varieties tolerated a longer period of moderate water deficiency, though to slightly different extents. The responses of Magma and Hereward suggested the initiation of degradation processes, showing that they had poorer stress tolerance. The examination of the photosynthetic apparatus thus confirms the conclusions drawn from the changes in peroxidase activity and from the fresh and dry mass data. The variety Magma, bearing the 1B/1R translocation, again responded differently to the other two varieties with the 1B/1R translocation.

The results presented were obtained by testing young wheat seedlings. However, regulational changes in the chosen physiological parameters occur equally in both seedlings and adult plants. Moreover, under moderate water stress conditions, changes in the activities of O_2 scavenging enzymes and in that of the thylakoid structure became more and more pronounced with time, facilitating the differential diagnosis of varieties differing to only a slight extent with respect to drought tolerance in long-term laboratory experiments.

Among the parameters chosen for the determination of drought tolerance, the measurement of PER activity could be a useful method for a rapid preliminary test, while the determination of the amounts and ratios of pigment-protein complexes could be used to determine whether the variety is capable of adapting to or surviving stress conditions. For the wheat varieties tested the stress tolerance estimated in the analysis agreed by and large with the results of field studies on adult plants. Further experiments on a wider scale will be required to determine whether the method could be applied to other wheat varieties or possibly to other cereals.

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SYNAPTONEMAL COMPLEX FORMATION IN HAPLOID WHEAT (*TRITICUM AESTIVUM*) AND IN WHEAT-RYE HYBRIDS WITH AND WITHOUT THE *Ph* GENE

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Using the microspreading technique, the formation of synaptonemal complexes (SC) was analysed in haploid plants of common wheat cv. Orofen obtained by anther culture and in F_1 hybrids between the mutant *ph1* or euploid of cv. Chinese Spring and rye cv. Pamirskaya. Both the haploid wheat plants and wheat-rye hybrids containing the *Ph1* gene were achiasmatic: only univalents were present at metaphase I (MI). In wheat-rye hybrid plants with a null dose of the *Ph1* gene, bivalents and multivalents with variable frequency were observed at MI. Extensive SC formation was revealed in prophase nuclei in the absence of the homologous partner for pairing. Synaptonemal complex formation was primarily initiated interstitially in wheat with a haploid chromosome set. The presence of the *Ph1* gene did not influence the ability to form SC between non-homologous chromosomes, but affected chromosome pairing stringency: in hybrid plants without the *Ph1* gene lateral elements tended to pair with themselves forming circular structures and bundles. The necessity of homology for an initiation of chromosome synapsis is discussed.

Key words: meiotic behaviour, synaptonemal complex, haploid, wide hybridization, *Ph1* gene, *Triticum aestivum*

Introduction

The synaptonemal complex (SC) has widely been accepted as a precise mechanism for the pairing of strong homologous chromosomes (Dawe, 1998). In diploids, the SC actually connects two homologous chromosomes in early meiotic prophase. There is a more complicated situation in allopolyploids where, besides the homologous partner for pairing, several pairs of partially homologous (homoeologous) chromosomes are present in the genome, creating competitive conditions for chromosome pairing. Therefore, a genetic control on diploid-like chromosome pairing is vital for the meiotic, and hence reproductive stability of sexually reproducing polyploids. There are strong indications of the genetic regulation of bivalent pairing in hexaploid and tetraploid wheats, hexaploid oats, tetraploid cottons and hexaploid tall fescue (Riley, 1960; Jauhar, 1975; 1977).

In polyploid wheat, the diploid-like chromosome pairing mechanism is principally controlled by the *Ph1* (*Pairing homoeologous*) gene that is located on the long arm of chromosome 5B (Sears, 1976; Jampates and Dvorak, 1986; Gill and Gill, 1991; Gill et al., 1993). The *Ph1* gene effect is manifested in the hemizygous condition in euhaploid wheat (2n=3x=21) or its hybrids with related species where only univalents are observed at MI. Haploid nullisomics for 5B

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(2n=20) have mean pairing of 7.5 univalents, 3.83 bivalents, 1.5 trivalents and occasional higher order associations (Riley, 1960). In nullisomic 5B wheat-rye hybrids there were 3.36 bivalents, 0.88 trivalents and 0.08 quadrivalents. A similar mean pairing is observed in wheat-rye hybrids where chromosome 5B has been substituted with chromosome 5D (Hutchinson et al., 1983).

In recent years there has been a considerable accumulation of data about SC formation in allopolyploids. In tetraploid (*Avena maroccana*) and hexaploid (*Avena sativa*) oats (Jones et al., 1989), only bivalent SCs are generated, while in tetraploid (*Triticum timopheevii*) (Martinez et al., 1996) and hexaploid (*Triticum aestivum*) (Hobolth, 1981; Jenkins, 1983; Holm, 1986) wheat, and triploid and tetraploid *Scilla autumnalis* (White et al., 1988; Jenkins et al., 1988) multivalent SCs are formed at zygotene, which are subsequently "corrected" to bivalents. "Correction" ensures bivalent pairing at the first metaphase.

A critical test of the effectiveness of this gene-regulated, diploid-like chromosome behaviour is provided by analysing the haploid complements of the polyploid species or their hybrids with related species. Because of an absence of competing homologous partners for pairing in the genome, haploids and interspecific hybrids are ideal for analysing synaptonemal complex formation between the homoeologous and non-homologous chromosomes. The aim of the present study was to investigate synaptonemal complex formation in wheat-rye hybrids with and without the *Ph1* gene and in trihaploid wheat.

Materials and methods

Haploid plants of wheat were obtained by the anther culture technique described by Wang (1988) with minor modifications. The hexaploid *Triticum aestivum* cv. Orofen was used as a donor. Spikes excised from the plants were sterilised by immersion in a 0.1% aqueous solution of "Belizna" containing chlorine for ten minutes. After three rinses in distilled sterilised water, the anthers were placed on Potato II medium. The embryoids were transferred to 190-2 differentiation medium. Plants derived from the embryoids were grown in a greenhouse. The chromosome number of the pollen plants was determined from the squashes of root tips dissected before the transfer of the plants to a greenhouse. Plants with 21 chromosomes were used for the ultrastructural analyses using the microspreading technique. Unfortunately, we failed to obtain haploids from the mutant *ph* wheat by anther culture: they perished before the tillering stage.

The *ph1* mutant *T. aestivum* cv. Chinese Spring (Sears, 1977), kindly provided by Dr. O. Maystrenko, and *Ph1* euploid Chinese Spring were crossed as the female with diploid rye *Secale cereale* cv. Pamirskaya to produce intergeneric hybrids. Immature spikes of F_1 hybrid plants were dissected and anthers containing pollen mother cells (PMCs) were used for electron and light microscopic examination.

Whole-mount spreading of prophase meiotic nuclei was made according to the protocol described by Gillies (1981) with minor modifications. The anthers of haploid and hybrid plants were placed onto Eagle's medium containing 0.1% bovine serum albumin and 2mM EDTA (pH 7.7) and sectioned with a razor blade; the anther contents were squeezed out with forceps. The cell suspension obtained was homogenized with an automatic pipette and dropped onto a hypophase surface (0.2 M sucrose solution on paraffin-polished glass). After 1.5–2 min, the spread nuclei were picked up by touching the hypophase surface with a glass slide coated with plastic film (0.6% solution of Falcon Petri dish dissolved in chloroform). The cell suspension distributed on the slide was fixed in formalin vapours for several hours. Thereafter the slides were air dried, washed in distilled water and stained with a 70% solution of $AgNO_3$ in a moisture chamber at 60°C for 2 hours. Film pieces containing well-spread nuclei were cut out and put onto single-hole grids. The spreads of meiotic nuclei were examined with a Tesla-500 electron microscope.

Chromosome preparations from PMCs at the first meiotic metaphase (MI) were prepared as described earlier (Enno et al., 1998). Every PMC was scored for the presence of univalents, bivalents and multivalents.

Results

Trihaploid wheat

Seventeen nuclei ranging from leptotene to late diplotene were analysed using the microspreading technique. A distinction between the prophase stages was made according to the degree of synapsis and the arrangement of chromosome telomeres. Leptotene nuclei in trihaploid wheat were morphologically similar to those in hexaploid wheat – the lateral elements (LEs) were fully developed before synapsis initiation. The bouquet configuration was also distinct in spread nuclei of trihaploid wheat, as most of the telomeres were located in a small area near the nucleolus. Synapsis started simultaneously at a large number of sites at a stage morphologically comparable with zygotene. Within the same nucleus, SC fragments were far more extensive in interstitial parts of the chromosomes than in the telomeric parts (Fig. 1a, b). The simultaneous presence of the long interstitial and the short telomeric paired fragments might indicate that in trihaploid wheat SC formation was primarily initiated interstitially. Synaptonemal complex fragments combined with lateral elements folded back on themselves were detected in zygotene and pachytene nuclei (Figs 1a, 2a). Local asynaptic regions with LEs of inequal length were present in the early pachytene nuclei (Fig. 2b). The complexes formed between the homoeologous or the non-homologous axial elements appeared to be normal: the distance between the lateral elements was indicative of the SC formation. Synaptonemal complex formation between homoeologous chromosomes appeared to be extensive in the cv. Orofen haploid genotype. At a stage comparable with pachytene, more than half the chromosome length was found to be synapsed in most nuclei. In several of the pachytene nuclei, the SCs were almost fully formed with the exception of asynapsed regions at the sites of pairing partner exchanges (Fig. 2c). Pairing partner exchanges involving the lateral elements of different SCs were found in zygotene, pachytene and diplotene nuclei (Fig. 3a, b, c).

Degradation of the unpaired lateral elements and the SCs was initiated in a few regions, generating a large number of fragments. Gaps occurred in one of the two lateral elements of SC (Fig. 4a). Unpaired LEs were primarily eliminated, while the SC segments appeared to be more resistant and primarily degraded from the ends rather than interstitially. The degradation was accompanied by an increase in the width and density of the lateral elements of the retained SC fragments. In some cases one of the two lateral elements had higher electron density than the other (Fig. 4b). Late diplotene nuclei were characterized by the appearance of short, distinct SC segments (Fig. 4d). Multivalent SCs were maintained at this stage (Fig. 3c). There were regions in several SC fragments where LE split into two (Fig. 4c).

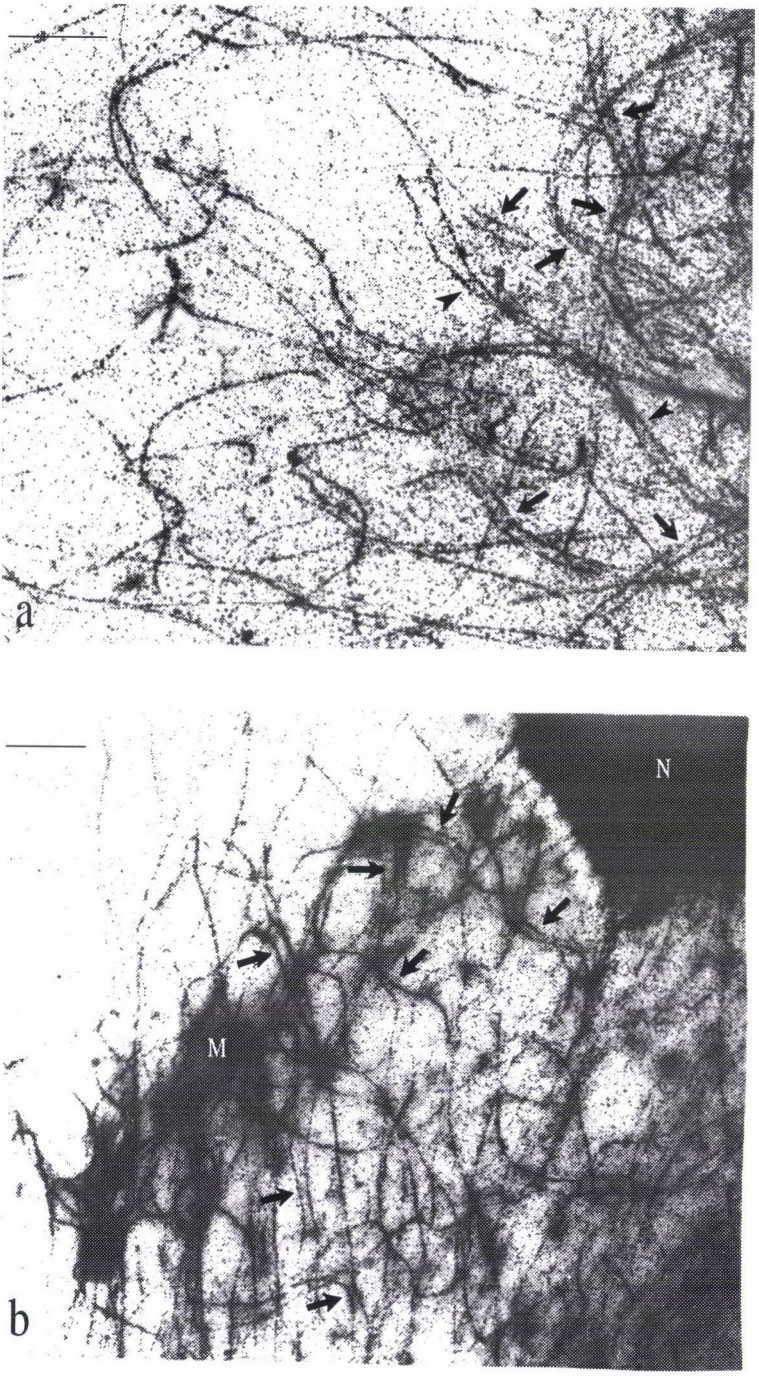


Fig. 1. Fragments of zygotene spread nucleus of trihaploid wheat: (a) interstitial SC fragments (arrows), fold-back paired loop (arrowhead); (b) paired LEs (arrows) at chromosome telomeres near nuclear membrane. Bars = 1μm

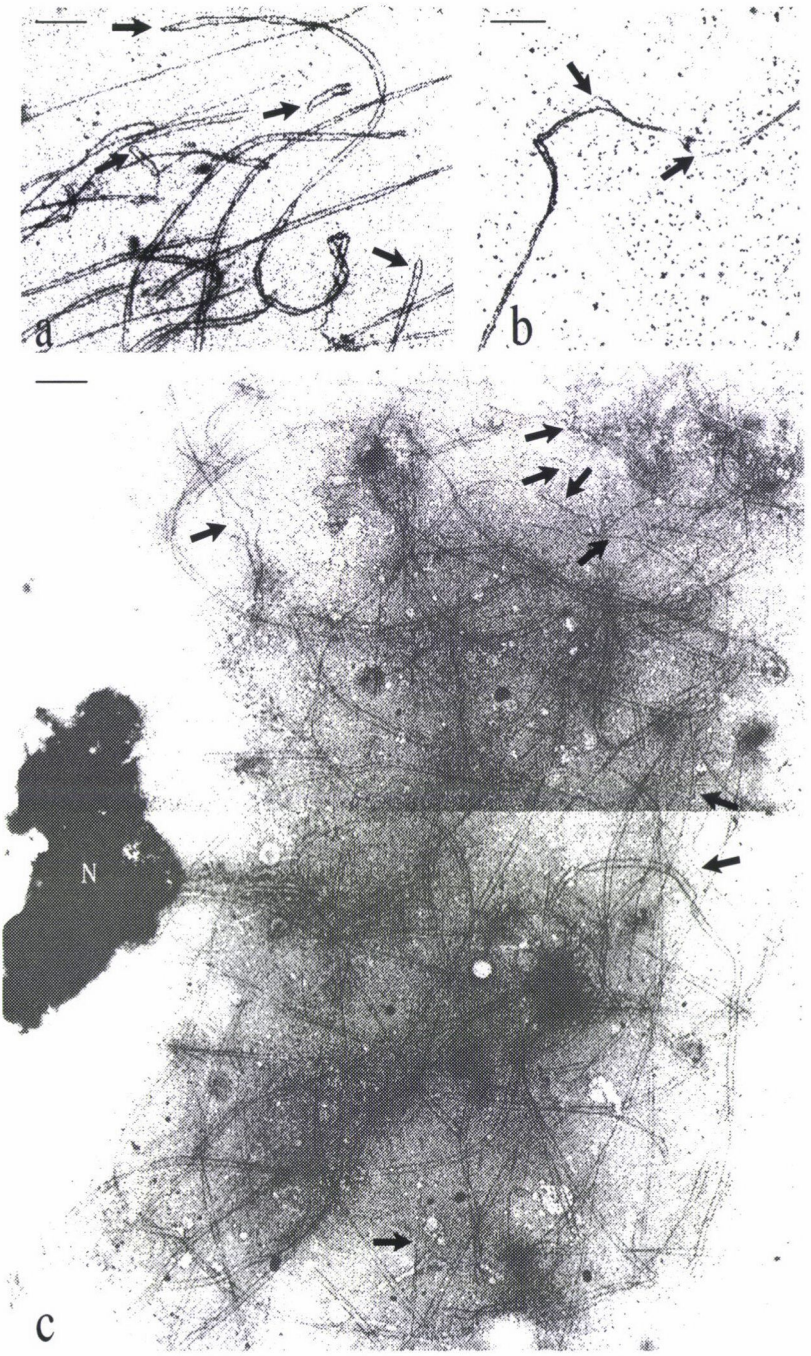


Fig. 2. Pachytene spread nuclei of trihaploid wheat: (a) LEs folding back on themselves (arrows); (b) inequality in the length of asynapsed LEs (arrows); (c) whole pachytene nucleus: asynapsed regions denoted by arrows. Bars = 1 μm

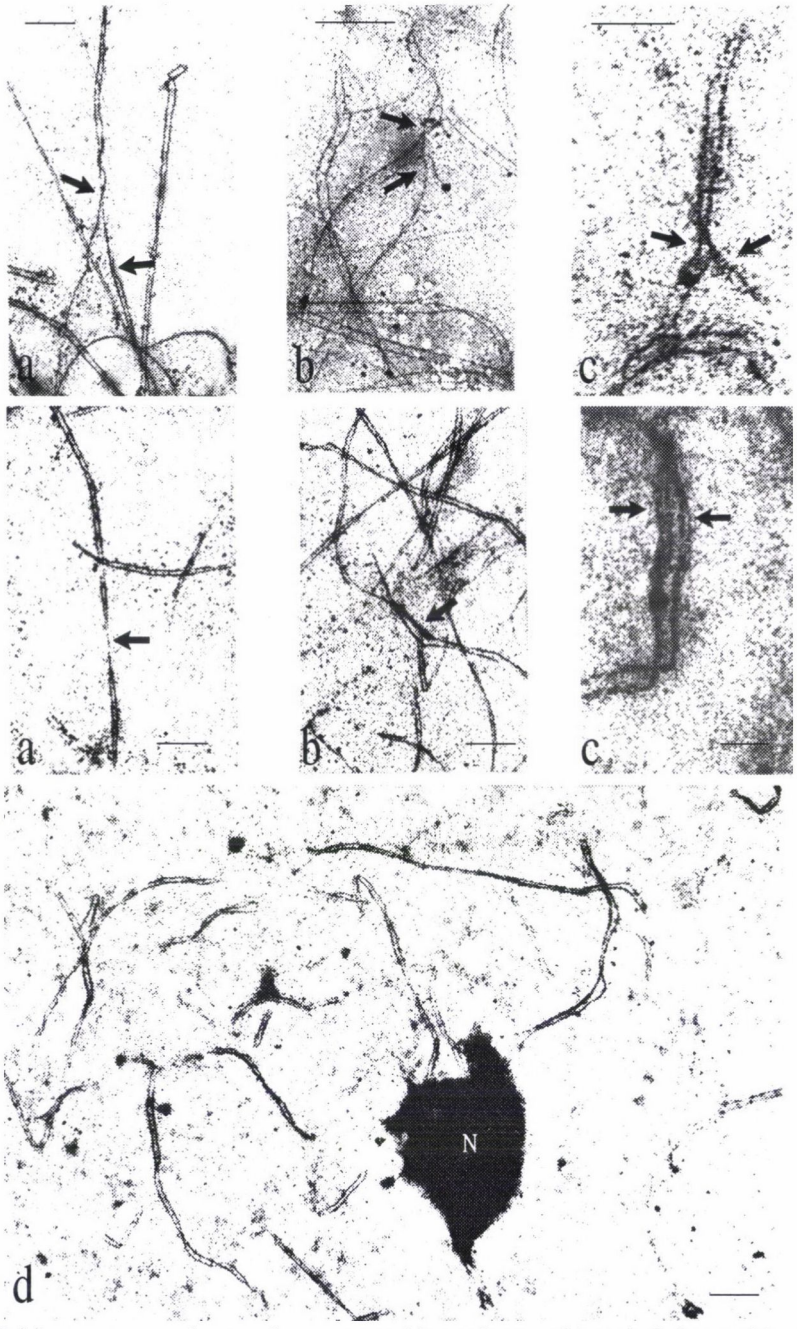


Fig. 3. Pairing partner exchanges in zygotene (a), pachytene (b) and diplotene (c) nuclei of trihaploid wheat (arrows). Bars: 3a,b = 1μm; 3c = 2 μm

Fig. 4. Fragments of the diplotene spread nuclei of trihaploid wheat: (a) gap in one of the two LEs (arrow); (b) inequal thickening of the LEs (arrow); (c) splitting of the LEs (arrows); (d) retained SC fragments, N: nucleolus. Bars: 4a,b,d = 1μm; 4c = 5 μm

Light microscopic observations of meiotic metaphase I showed that all trihaploid plants were achiasmatic: only univalents were observed (data not shown).

Wheat-rye hybrids

Nineteen nuclei of the F_1 hybrids involving the *ph1* mutant and twenty-five nuclei of F_1 hybrids with euploid wheat were analysed by spreading microsporocytes on a hypotonic surface. Electron microscopic investigations revealed the presence of morphologically normal SCs in wheat-rye hybrids carrying both the normal *Ph1* allele and the mutant *ph1* allele. Chromosome pairing was incomplete in both hybrid genotypes. It was apparent that no major difference as to the degree of synapsis exists between the two hybrid genotypes (Figs 5a, 6). The identification of the individual lateral elements or their assignment to the rye genome or the wheat genomes was not attempted. Therefore, it was not possible to determine the individual chromosomes involved in synapsis. Synapsis was asynchronous in wheat-rye hybrid nuclei: there was a substantial variation in the degree of synapsis between different paired chromosomes. Fragments of interstitial synapsis located between the asynaptic regions were regularly detected in zygotene and pachytene nuclei of hybrid plants (Figs. 5b, c, d). Inequality in the length of asynapsed LEs was observed in several regions of asynapsis (Fig. 5d). Synaptonemal complex fragments combined with lateral elements folding back on themselves were found in zygotene and pachytene hybrid nuclei (Fig. 5c, e). Pairing partner exchanges involving the lateral elements of different SCs were found in wheat-rye hybrid genotypes both with and without the *Ph1* gene. However, in *ph1* mutant wheat-rye hybrid plants, lateral elements tended to fold back and to pair with themselves, forming circular structures or bundles (Fig. 5e). These structures were never observed in euploid wheat-rye hybrids.

Light microscopic analysis revealed significant differences in meiotic chromosome behaviour between hybrids with and without the *Ph1* gene. In F_1 euploid wheat-rye hybrids, the asyndetic type of meiosis was observed with the prevalence of 28 univalents at MI (Fig. 7a). The mean number of bivalents and univalents per cell was 0.2 and 27.6, respectively (Table 1). The use of Chinese Spring mutant *ph1* in a cross with rye showed a significant increase in homoeologous chromosome pairing (Fig. 7b) with a mean bivalent number of 4.9 per cell. The percentage of PMCs with multivalents was 44.1 (Table 1).

Table 1
Chromosome pairing at MI of meiosis in wheat × rye F_1 hybrids using mutant *ph1* of Chinese Spring

Hybrid combination	No. of PMCs observed	Average number per PMC at MI				% PMCs with multivalents
		Univalents	Bivalents	Multivalents	Chiasmata	
CS × Pamirskaya	670	27.6 (22–28)	0.2 (1–3)	0	0.2	0
ph1 × Pamirskaya	419	16.3 (7–27)	4.9 (1–10)	0.54 (1–3)	9.2	44.1

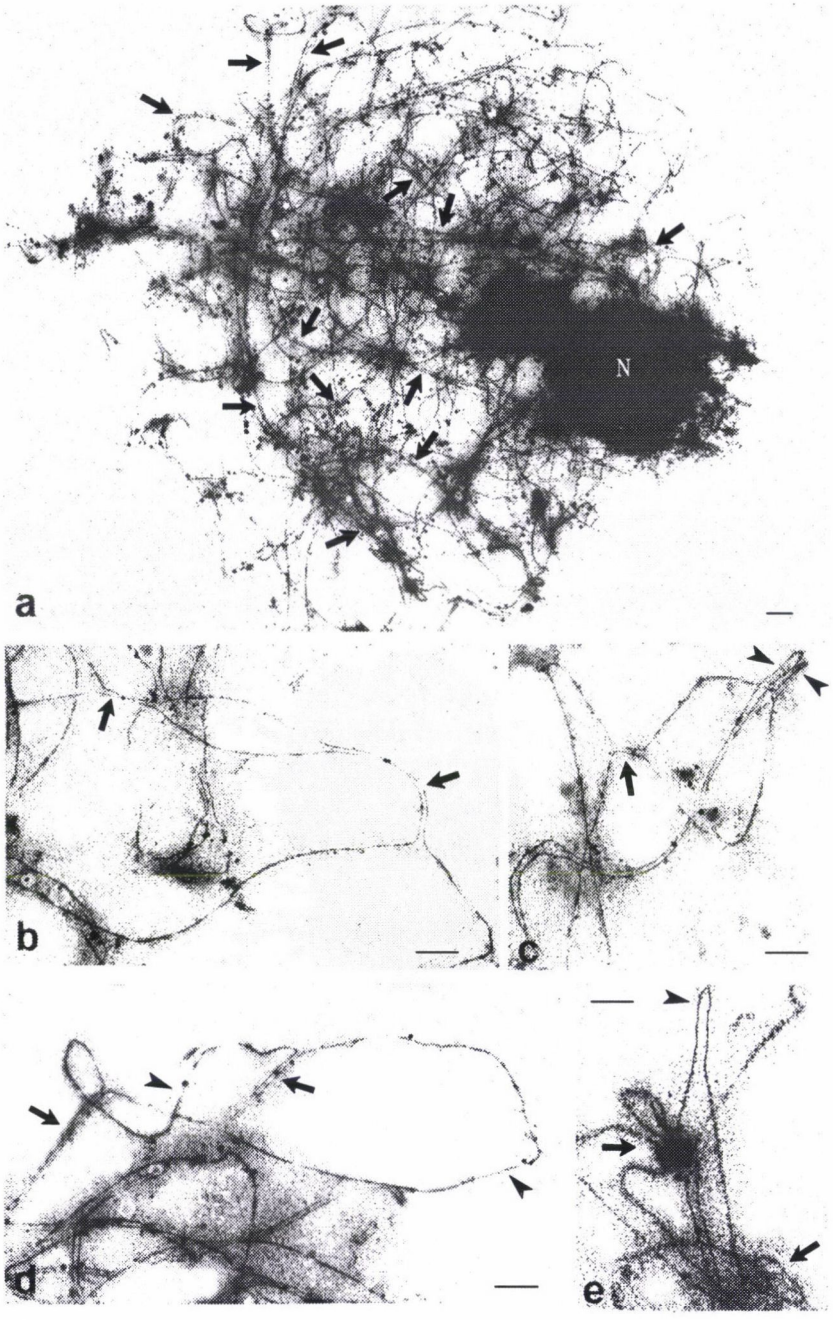
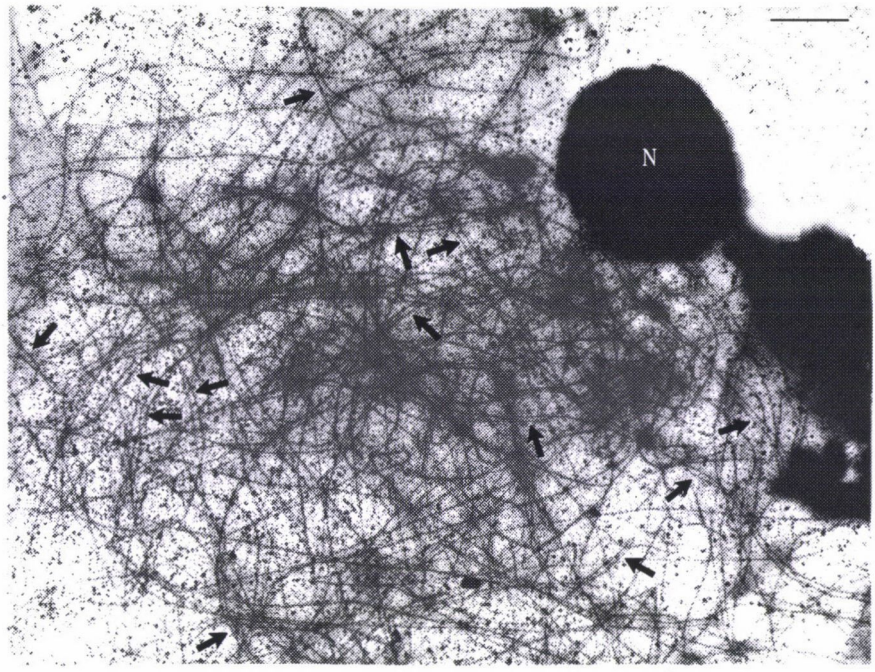


Fig. 5. Zygotene spread nucleus of the F_1 hybrid between mutant *ph1* and rye: (a) whole nucleus: SCs are denoted by arrows; (b–e) fragments of the same nucleus: (b–d) regions of interstitial synapsis (arrows) between the asynaptic regions; (c, e) LEs folding back on themselves (arrowheads); (d) inequality in the length of asynapsed LEs (arrowheads); (e) bundles (arrows). Bars = 1μm



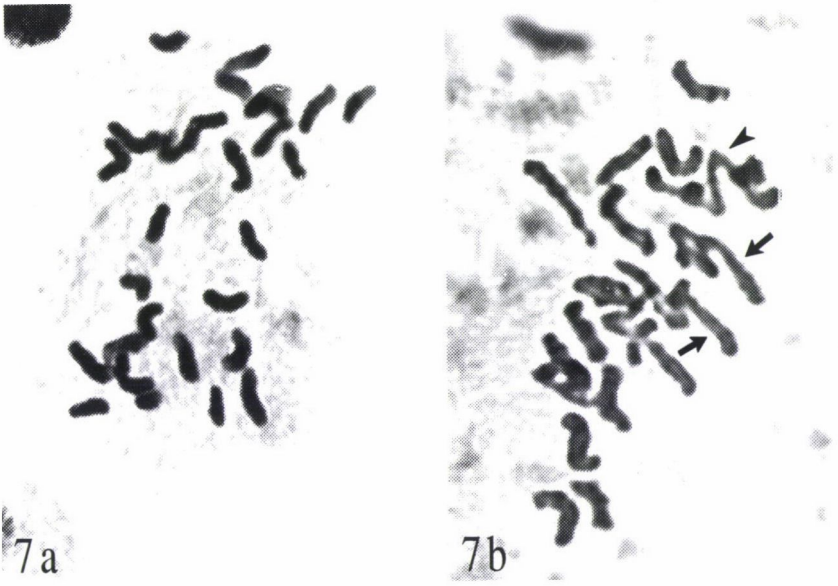


Fig. 6. Zygotene spread nucleus of the F_1 hybrid between euploid wheat and rye: SCs are denoted by arrows. Bar = 1μm

Fig. 7. Metaphase I in F_1 wheat–rye hybrids: (a) 28 univalents in the hybrid with euploid wheat; (b) three bivalents, two trivalents (arrows), one tetravalent (arrowhead) and twelve univalents in the hybrid with mutant *ph1*

Discussion

The occurrence of SCs in haploid wheat prophase I nuclei was not unexpected in the light of similar findings in other haploids (Menzel and Price, 1966; Sen, 1970; Ting, 1973; Gillies, 1974; Loidl et al., 1991; de Jong et al., 1991), where apparently normal SCs were formed between non-homologous chromosomes of one and the same haploid set. As these electron microscopic observations were limited to single SC segments from sectioned midprophase nuclei, the question arose as to whether the segments of SC reported in haploids involved small homologous duplicated regions in otherwise non-homologous chromosomes. Although homoeologous chromosomes from closely related basic genomes in common wheat have even more homology to pair and to form SCs than non-homologous chromosomes of tomato, barley or maize, the almost complete synapsis of the common wheat haploid genome makes it unlikely that the observed SC formation is based on extensive homology. A mechanism recognizing homologues was shown to operate independently of the SC (Schwarzacher, 1997). The presynaptic alignment of homologous chromosomes revealed prior to chromosome synapsis in wheat (Timofeyeva et al., 1988) is a cytological manifestation of this mechanism. The fact that in haploid wheat, synapsis is initiated in the interstitial regions of the chromosomes, while in euploid wheat, SC formation starts in the chromosome telomeres (Timofeyeva et al., 1988) provides evidence that SC initiation may occur in the same species at various sites along the chromosomes. Moreover, the appearance of new synapsis initiations during the progression of synapsis simultaneously with the extension of existing SCs indicates that synapsis initiation is not synchronised in the nucleus. Therefore, it is unlikely that special sites exist along the chromosomes for the initiation of chromosome synapsis. It is more likely that any chromosomal region, at least on the cytological level, can be a potential SC initiation site and that SC initiation can start at any site where the axial elements have approached each other up to the critical distance. The telomeric SC initiation observed in euploid wheat does not thus seem to be the result of the abundance of any presumptive specialised sites for SC initiation, but only reflects the closer arrangement of homologous chromosomes on the nuclear envelope. The spatial relationship of homologues within the nuclear volume (Schwarzacher et al., 1989; Schwarzacher, 1997) and/or a certain location of telomeres belonging to homologues on the nuclear membrane (Scherthan et al., 1996) probably ensures pairing only between homologous chromosomes. The absence of homologues seems to remove the constraint of matching homologous sites (perhaps a prealignment condition) and allows SC formation by chance collisions of lateral elements. The random process of SC initiation confirms the hypothesis that synapsis is not initiated by the matching of unique sites in the DNA (Loidl, 1990) and, consequently, SC initiation does not require homology. Recently it was shown that the initiation of synapsis in haploid yeast requires double-strand breaks, but not homology (Gilbertson and Stahl, 1994).

A comparison of synaptonemal complex behaviour in wheat-rye hybrids carrying the *Ph1* allele or the mutant (null) *ph1* allele indicates that the *Ph1* gene does not control the ability to form SC between non-homologous chromosomes: no major difference as to the degree of synapsis exists between the two hybrid genotypes. The formation of circular structures and bundles from axial elements indicates that the *Ph1*gene affects the stringency of chromosome pairing.

The lack of recombination in both wheat haploids and wheat-rye hybrids carrying the *Ph1* gene cannot be attributed to asynapsis, as extensive synaptonemal complex formation between non-homologous chromosomes is found in prophase nuclei of both genotypes. The presence of univalents at metaphase I in haploid plants and in wheat-rye hybrids carrying the *Ph1* gene, on the one hand, and the formation of bivalents and multivalents in hybrids carrying the *ph1* allele, on the other hand, indicate that this gene suppresses recombination between non-homologous chromosomes. These data support the hypothesis on the dual effect of a null mutation: a reduction in synapsis stringency and the permission of recombination between non-homologous chromosomes (Gillies, 1987). Recent investigations using genomic *in situ* hybridization showed that the *ph1* allele also influences chromatin organisation in both meiotic and premeiotic nuclei (Mikhailova et al., 1998). The authors concluded that the *Ph1* locus regulates not only meiotic chromosome pairing and synapsis, but also regulates the premeiotic associations of homologues.

It is assumed that the rye genome strongly promotes homoeologous pairing in hybrids with wheat. In the present investigation, the SC formation was more extensive in haploid wheat cv. Orofen than in hybrids between wheat cv. Chinese Spring and diploid rye cv. Pamirskaya. Wang and Holm (1988) have demonstrated that in hybrids between wheat cv Chinese Spring and rye cv. Ptili K 135, up to 40–50% of the complement paired with a synaptonemal complex, while the mean degree of synapsis achieved in haploids of cv. Chinese Spring amounted to 35–40% (Wang, 1988). However, in haploids from cv. Kedong up to 90% of the complement was combined with SCs (Wang, 1988). This is comparable with the degree of synapsis observed in haploids from cv. Orofen. It has also been shown that each species or cultivar of rye could behave differently in homoeologous pairing (Cuadrado and Romero, 1988). The mean number of bivalents and univalents in hybrids with rye cv. Pamirskaya was 0.2 and 27.6, respectively, while in hybrids with cv. Petkus these figures were 0.3 and 27.4. In the presence of the *ph1* mutation there were on average 4.9 bivalents and 0.54 (1-3) multivalent associations per cell in hybrids with rye cv. Pamirskaya, while in hybrids with cv. Petcus the corresponding numbers were 4.6 and 0.46 (1-3), and with rye self-fertile line Kc-517/8 5.7 and 0.94 (1–4), respectively (Shnaider and Priilinn, 1984; Shnaider, 1987). Thus, in hybrids, the control of meiotic pairing can be strongly modified by interaction between the wheat genetic system and the genes of related species.

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A FASCIATED MUTANT IN COWPEA (*Vigna unguiculata* (L.) Walp.)

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A fasciated cowpea line resulting from spontaneous mutation in the F_4 population of a backcross involving TVU 3000 × IT82D-60[4] was studied. The mutant plants were both male and female sterile and exhibited crumpled petals and sepals, rosette branching and also an abnormal number of stigmata ranging from zero to two. Fasciation was also associated with other aberrant characteristics. Plant height at maturity was 64.10 cm and 42.50 cm for the normal and fasciated mutant, respectively. Fasciated mutants produced more fodder (24.9 g/plant compared to 16.4 g/plant for normal plants). Inheritance studies indicated that fasciation was conditioned by a single recessive gene, which is being designated as 'fa'. Pollen sterility was caused by the sudden degeneration of tapetal cells resulting in the starvation of the microspores. The implications of the fasciated mutant for cowpea improvement are discussed.

Key words: *Vigna unguiculata* (L.) Walp, stem fasciation, sterility, mutant, recessive gene

Introduction

Fasciation of the stem has been reported in a number of crop species such as pepper (Wilson et al., 1982; Rajam and Subhash, 1995), greengram (Singh, 1981), gardenpea (Gottschalk and Wolff, 1983) and soybean (Palmer and Kilen, 1987). In all cases, fasciation involves a change from the normal round or polygonal stem or axis to one that is flat-banded or ribbon-shaped (White, 1948; Gottschalk, 1979). Fasciation of the stem is often combined with the formation of an increased number of flower buds and flowers which accumulate in the top region of the plants (Gottschalk and Wolff, 1983). Stem fasciation is often associated with reproductive abnormality, such as abnormal floral buds with reduced sizes, abnormal anthers, pollen sterility and rosette branching (Singh, 1981; Wilson et al., 1982, Rajam and Subhash, 1995).

The genetic basis of stem fasciation has been studied in several crops and seems to be simply inherited (Wilson et al., 1982). A great deal of work has been done in gardenpea, in which different mutants have shown different genetic segregation patterns ranging from monogenic recessive (Marx and Hagedorn, 1962) two polymeric genes (Lamprecht, 1952) or three major genes supplemented by several modifiers (Sidorova, 1970), to complicated segregations (Gottschalk, 1979; Rod and Vagaerova, 1970). In pepper, stem fasciation is reported to be caused by a single recessive gene (Wilson et al., 1982; Rajam and Subhash, 1995).

Among cultivated plants, this trait has been of practical value in scientific or genetic studies. Fasciation of the stem is agronomically utilized in gardenpea

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(Gottschalk and Wolff, 1983; Gottschalk, 1979). Since fasciation is inherited as a recessive character, it could be used as a gene marker in cowpea improvement programmes. The literature available on cowpea does not reveal any report on stem fasciation. The purpose of this paper is to describe a fasciated mutant in cowpea (*Vigna unguiculata* (L.) Walp).

Materials and methods

The materials for this study originated from a spontaneous mutation in the backcross population of TVU 3000 × IT82E-60[4]. One of the F_3 progenies, IT84E-1-176, showed 8 normal plants and 7 sterile plants, which had fasciated stems and other abnormalities associated with it. All the 8 normal plants were individually harvested for further genetic studies. The progenies of these plants were tested in two successive generations for segregation patterns and cytological studies were also conducted. Fertile plants were distinguished from sterile plants on the basis of (i) deformed anthers; (ii) small pollen mass as well as abnormal pollen grains; and (iii) plant morphology. Plants showing fasciated stems were tagged before the onset of flowering and later examined for pollen and anther characteristics. These plants remained green with leathery leaves when the normal plants had fully matured and dried. The following data were collected on twenty plants: (i) plant height at maturity; (ii) petal length and width; (iii) number of anthers; (iv) anther length; (v) number of pistils/flower; (vi) pod length; (vii) grain yield and components of grain yield; and (viii) straw/fodder weight/plant. The ratio of male sterile to male fertile plants within each segregation progeny row was recorded at maturity. The goodness-of-fit to different genetic ratios for fertile vs. sterile segregates within each row was determined by chi-square tests.

Young flower buds from normal and fasciated plants were fixed in acetic acid-ethanol (1:3 v/v) fixative and acetocarmine smears were prepared. Pollen grains were mounted in 1% acetocarmine to determine pollen fertility at a magnification of × 400 using an ocular micrometer. The number of pollen grains per anther was determined by carefully removing them from the anther lobes with dissecting needles. Acetocarmine smears were prepared and a glass cover divided into 4 quadrants placed on the smear. The number of pollen grains in each quadrant was counted and added to get the total number per anther. Pollen size was measured as the length of the base of the rectangularly-shaped pollen. Pollen stainability as an index of pollen fertility was determined using 1% acetocarmine solution. The pod setting potential of the mutant was studied by allowing natural out-crossing in addition to carrying out one hundred and thirty hand pollinations. Flower buds of the mutant were fixed in 1:3 acetic acid-alcohol (v/v) and meiotic studies were carried out employing the acetocarmine squash technique. Microtome sectioning of anthers from fasciated and normal plants was carried out to study microsporogenesis and anther development following the method described by Beryln and Miksche (1976).

Results and discussion

Morphological description

Plants with fasciated stems were determinate in growth habit, with thick, dark green leathery leaves which remained attached to the stem even at the time when normal plants were already showing pod ripening. The fasciated plants were shorter than normal plants of the same genotype (Table 1). In these plants with fasciated stems, almost all the primary branches arose by termination of the flat stem into a conspicuous rosette form of branching. These plants flowered at the same time as others in the line, but the flower buds were smaller. Individual flowers on the normal plants in this line had 5 petals, 5 sepals, a total of 10 stamens, and a pistil, whilst the

fasciated plants had from 3 to 5 petals (most of which were crumpled) and 7 to 13 stamens with a mean of 9.5 ± 1.1. Some of the anthers were sessile, as they were directly connected to the base of the wings. About 20% of the flowers observed on these plants had no stigma, whilst 5% had double stigmata. On top of some of the flattened stems a large number of clustered flower buds was found. The flower buds on fasciated plants opened at anthesis. One hundred and thirty crosses made in both directions failed to set pods. The mean grain and fodder yield, and the yield components of the normal and fasciated mutants are indicated in Table 2. The normal plants produced an average of 15.7 pods per plant and 9.8 seeds/pod, whilst the mutant produced no pods or seeds. Grain yield per plant for the normal plant was 12 g. It is worthy of note that the fasciated mutant produced more fodder than the normal plants. This is expected, since no pods or seeds were produced. All the photosynthate was channelled into vegetative production. The fasciated mutant may be useful as fodder for livestock, especially during the dry season when livestock feed is not available, particularly in the savanna agro-ecologies in West Africa.

The inability of the fasciated mutant to produce pods and seeds indicated that the fasciated plants may be both male and female sterile. In greengram, stem fasciation was reported to be associated with complete sterility (Singh, 1981), as was observed in the present study.

Mean pollen number, anther size and stainability

The mean pollen number per anther, size and stainability are reported in Table 3. Variable pollen/anther was observed for both fasciated and normal plants. The normal fertile plants had more pollen grains per anther than fasciated sterile plants. The percentage pollen stainability for fasciated sterile plants was 44.6, whilst the normal fertile plants gave 95.8%, implying that normal plants were more fertile than fasciated plants. The inability of stained pollen grains from fasciated plants to set pods implies that other factors might be responsible for sterility. This observation agrees with work done by Rajam and Subhash (1995).

Table 1
Mean vegetative and floral characteristics of a normal and fasciated cowpea mutant IT 85D-3624

Plant characteristic	Normal	Fasciated
Plant height at maturity (cm)	64.10 ± 7.0	42.50 ± 9.0
Petal length (cm)	2.65 ± 0.2	2.00 ± 0.4
Petal width (cm)	1.90 ± 0.1	1.26 ± 0.3
No. of anthers	10.00 ± 0.0	8.71 ± 0.8
Anther length (mm)	1.40 ± 0.03	0.89 ± 0.05
No. of pistils	1.00 ± 0.00	1.30 ± 0.01

Table 2
Yield and yield components of normal and fasciated cowpea mutant IT85D-3624

Yield and yield components	Normal	Fasciated
Pod length (cm)	17.0 ± 1.6	0.0
No. of seeds/pod	9.8 ± 0.6	0.0
No. of pods/plant	15.7 ± 1.8	0.0
Grain yield/plant (g	12.4 ± 2.0	0.0
Straw weight/plant (g)	16.4 ± 2.9	29.9 ± 4.1

Table 3
Mean number of pollen grains/anther, pollen size and percentage stainability in a normal and fasciated cowpea mutant IT85D-3624

Parameter	Normal	Fasciated
Mean no. of pollen grains/anther	694 ± 31	391 ± 24
total no. of pollen grains	1420 ± 98	921 ± 71
No. of stained pollen grains	1368 ± 82	596 ± 48
No. of unstained pollen grains	60 ± 5	325 ± 16
Percentage pollen stained	95.8	64.7
Pollen size (μm)	70 ± 6	34 ± 9

Inheritance

The original F_3 progenies had 8 normal and 7 fasciated plants, which was much closer to the 9:7 ratio than the 3:1 ratio. However, the progeny tests of normal plants indicated a monogenic segregation. Of the 8 normal progenies, 3 bred true for normal plants while 5 progenies segregated into normal and fasciated in proportions of 11:4, 19:5, 21:3 and 20:5, some of which fitted closely to a 3:1 ratio. Owing to the inconsistencies in the genetic ratios, twenty-four randomly selected fertile plants from segregating progenies were tested. The segregating pattern in the individual progenies are given in Table 4, which indicated a close fit to the 3:1 ratio with no heterogeneity. Thus, the inheritance of stem fasciation is controlled by a single recessive gene, which agrees with the results of inheritance studies conducted by Marx and Hagedorn (1962) in *Pisum sativa* and Wilson et al. (1982) in *Capsicum annuum* (L). The recessive gene controlling fasciation was found to be associated with sterility and floral modification of the plants in IT85D-3624 (a renaming of IT84E-1-176). A similar observation was made by Bergh and Lippert (1964) in pepper who attributed this to either induced physiological changes which may have modified the pathway of both floral and vegetative organs or to chromosomal aberrations causing inactivation or loss of many genes.

Cytological study

Various cytological irregularities of the fasciated plants were observed, but none resulted in an increase in the number of chromosomes (2n=22). This agrees with cytological observations in peas and tobacco (White, 1948: Kaul, 1988). Within

the same flower bud, some pollen mother cells showed meiotic abnormalities, while others were normal. Microtome sections of anthers from fasciated plants revealed that the tapetum had degenerated; consequently, the developing spores shrivelled. The continued degeneration of the tapetal cells resulted in misshapen pollen grains. The significance of the tapetal layer for the nourishment and proper development of microspores has been emphasized (Heslop-Harrison, 1970; Kaul, 1988).

The basic cause of fasciation is a disturbed metabolism involving excessive nutriment, which mobilizes energy that must be utilized (Wilson et al., 1982). This energy, once accumulated, must go into growth and it becomes wildly expended in abnormal and unpredictable tissue production, generally to the detriment of the plant (White, 1948; Gottschalk, 1979). From the literature, it appears this is the first time stem fasciation has been reported in cowpea. Fasciation of the stem is agronomically utilized in gardenpea, where a lot of work has been done on stem fasciation (Gottschalk and Wolff, 1983; Gottschalk, 1979). Since stem fasciation is simply inherited, as indicated in the present study and in earlier studies in pepper by Wilson et al. (1982) and in gardenpea by Marx and Hagedorn (1962), it could be used as a marker for genetic studies in cowpea. The continuous growth of the fasciated mutants after anthesis makes them useful as fodder for livestock especially during the dry season where lack of proteinaceous feed is a major problem in the Guinea and Sudan savannas of West Africa. Inheritance studies indicated that stem fasciation was conditioned by a single recessive gene, which is being designated as 'fa'.

Table 4

Frequency of fasciated and normal plants in the segregating progenies of IT85D-3624

Family	Frequency of plants		$\chi^2(3:1)$	Probability
	Normal	Fasciated		
1	29	11	0.13	0.50–075
2	50	18	0.08	0.75–0.90
3	31	14	0.90	0.25–0.50
4	40	15	0.15	0.50–0.75
5	27	9	0.00	0.95–0.99
6	26	8	0.04	0.75–0.90
7	20	10	1.11	0.25–0.50
8	48	15	0.05	0.75–0.90
9	51	16	0.04	0.75–0.90
10	20	9	0.56	0.25–0.50
11	33	10	0.07	0.75–0.90
12	25	8	0.01	0.90–0.95
13	30	9	0.08	0.75–0.90
14	26	12	0.88	0.25–0.50
15	30	11	0.07	0.75–0.90
Pooled	486	175	0.77	0.25–0.50
Heterogeneity	–	–	3.40	P>0.99

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EFFECT OF CONCOMITANT OVULE CULTURE ON ANTHER CULTURABILITY IN WHEAT AND WHEAT × WHEATGRASS WIDE CROSSES

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Anther culture is a useful method to produce doubled haploids from pollen. It also holds potential in producing addition, substitution and translocation lines from wide crosses. However, low callus induction and green plantlet regeneration limit the routine application of anther culture in wheat. In an effort to improve anther culturability, the effect of co-culture of ovules with anthers on solid media was studied in six wheat genotypes and three wheat × wheatgrass backcrosses. Wheat genotypes differed significantly for callus induction as well as plantlet regeneration and the trend remained the same in anther culture as well as in co-culture. Co-culture of wheat anthers with ovaries did not improve their response. The anther culturability ranking of the previously screened wheat cultivars remained the same in co-culture. The frequency of albino plants was high without as well as with ovules. In wheat × wheatgrass backcrosses, co-culture indicated the beneficial effect of ovules on anther culturability.

Key words: anther culture, anther ovule co-culture, wheat, wide crosses

Introduction

Research on wheat anther culture expanded greatly after Craig (1974) obtained haploids from common wheat (*Triticum aestivum*) anthers. The technology is useful in wheat breeding, transformation and basic research (Baenziger et al., 1984; Liang and McHughen, 1987; Jahne and Lorz, 1995; Jain et al., 1996). It also holds potential in wide crosses for producing alien addition and substitution lines and for creating somaclonal variation in the form of translocation lines. However, the low frequency of callus induction and green plantlet regeneration are some of the main limiting factors in wheat (Navarro-Alvarez et al., 1994) as well as in wide crosses of wheat (Sharma et al., 1994). The anther culture response depends on wheat genotype and culture conditions (Jones and Petolino, 1987; Metz et al., 1988; Han-min et al., 1990; Lu et al., 1991; Karsai et al., 1993; 1994), so it is feasible to use genotypes with high callus induction and plantlet regeneration. However, the selection of such genotypes is needed and these may not be the best agronomic types. Thus, any *in vitro* modification to enhance anther culture response will be useful.

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Conditioning the liquid medium with ovules was advantageous for the directly isolated microspore culture of wheat (Datta and Wenzel, 1987; Hu and Kasha, 1997; Puolimatka et al., 1996; Patel and Darvey, 1998). Mejza et al. (1993) regenerated plants from isolated microspores using ovary co-culture but the procedure was complicated and labour-intensive. Our objective was to study the effect of the co-culture of ovules on anther culturability in wheat and wheat × wheatgrass wide crosses using solid medium and intact anthers, which is a simpler approach than using liquid medium and isolated microspore culture.

Materials and methods

The material consisted of six common wheat genotypes and five backcross populations of three common wheat × wheatgrass intergeneric hybrids. Among several wheat genotypes, good, intermediate and poor responders to anther culture (without ovaries) were identified (Lu et al., 1991). Three of the six wheat genotypes, Pioneer 2550, Cardinal and Caldwell, represented these good, intermediate and poor categories, respectively. The other three wheat genotypes, GK Élet, GK Góbé and GK Délibáb were from Hungary (Pauk et al., 1995) and had good to intermediate anther culture response (Z. Jekkel, personal comm.). Wheat × wheatgrass backcrosses included BC_1, BC_2 or BC_3 from hybrids between wheat and *Thinopyrum ponticum*, *Th. intermedium* or *Th. trichophorum* with wheat as the recurrent parent (Sharma et al., 1994).

In general, the procedure of Lu et al. (1991) was followed for anther culture. The seeds were planted in standard greenhouse flats, and the germinated seedlings were vernalized for 9 weeks. The plants were grown in pots having 10 cm diameter under an 8/16 h day/night photoperiod for 2 weeks and then a 16/8 h photoperiod. The temperature in the greenhouse was 20–22/16–18°C day/night. Spikes were harvested when their tips were at the junction of the sheath and blade of the penultimate leaf, which largely corresponds to mid- to late-uninucleate stage of pollen. The spikes were placed in flasks of water and kept in the dark at 4°C for one to three weeks before excising and culturing the anthers. Close to 70 anthers were inoculated aseptically per 100 × 15 mm Petri dishes containing 25 ml N6 medium (Chu, 1978; Lu et al., 1991). The actual number of anthers cultured in each dish was counted after culture. In dishes where the co-culture of anthers and ovaries was done, the anthers to ovary ratio was 3:1, and the anthers were surrounded by ovaries from the same spikes. For comparison, dishes without ovaries were also prepared. Callus induction was scored after 8-week incubation in the dark at 28°C. The calli were transferred to the regeneration medium and incubated at 12/12 h day/night photoperiod and 26°C. The regeneration medium was the same as that described by Chu (1978) with the modification that sucrose was reduced to 3% and 0.5 mg l^{-1} NAA was used instead of 2,4-D.

For wheat anther culture, the data were analysed as a completely randomized block design. Induction and regeneration frequencies were calculated as percentages of anthers callused and differentiated into plantlets, respectively. For callus induction and regeneration data that were subjected to ANOVA, $\sin^{-1}\sqrt{y/100}$ transformation was performed. When ANOVA was significant, the Student-Newman-Keuls (SNK) multiple range test was performed for mean separations using the Statistical Analysis System (SAS Inst., 1988).

Results and discussion

A total of 18,126 anthers of wheat, 7384 without and 10,742 with ovules, were cultured in 235 Petri dishes, excluding 18 dishes that became contaminated. Overall callus induction without and with ovules was 3.9% and 3.3%, while overall plant regeneration was 1.5% and 1.0%, respectively. Of these, 14,526 anthers (184 Petri dishes), 5642 without and 8884 with ovules,

were subjected to ANOVA, excluding those dishes where replications or all genotypes at a particular level were missing. The overall difference in callus induction and plant regeneration without and with ovules remained about the same, being 4.0% and 3.3% for callus induction and 1.6% and 1.1% for plant regeneration, respectively (Table 1). The overall trend in the callus induction and regeneration response of Pioneer 2550, Cardinal and Caldwell was the same as that found, without co-culture, by Lu et al. (1991). The more responsive genotypes (genotypes giving higher percentages of callus formation and plantlet regeneration) remained more responsive and the poor responder remained poor not only in anther culture alone but also in co-culture with ovules (Table 1). The Hungarian material showed responses in between the best and worst responders of the U.S. wheats. The main effects of co-culture were non-significant, indicating that culturing ovaries with anthers was not beneficial for anther culture response in the genotypes studied. Co-culture did not improve the response of intact anther cultures of microspores on solid medium. According to Hu and Kasha (1997), some essential factors may be supplied by ovaries for androgenesis. Apparently, the uptake or effect of such factors on microspores in intact anthers on solid medium was not significant in our study.

The wheat genotypes differed significantly for callus induction as well as for plantlet regeneration ($P<0.01$). Since genotypic main effects were significant, means were separated using the SNK multiple range test (Table 2). A comparison of the means confirmed the results of Lu et al. (1991) that Pioneer 2550, Cardinal and Caldwell represent three different anther culturability classes. Although a number of factors, including co-culture with ovules, incubation temperature and medium conditions, were different in the present study, these genotypes exhibited the same order as in Lu et al. (1991), so there is clearly a strong genotypic effect on anther culturability. Lazar et al. (1984) and Bruins and Snijders (1995) showed that a high proportion of the total variance for anther culturability was due to genotypic effects.

Table 1

Percentage callus induction and percentage regeneration from anthers of wheat genotypes in anther culture (A) and anther ovary co-culture (A+O)

Genotype	A or (A+O)	Anthers cultured	% callus induction	% regeneration
Pioneer 2550	A	1660	6.28	2.94
	A+O	2300	6.68	2.39
Cardinal	A	669	3.53	1.49
	A+O	1211	1.70	0.33
Caldwell	A	1658	0.86	0.27
	A+O	3310	0.87	0.18
GK Délibáb	A	957	5.50	2.50
	A+O	1275	4.31	1.84
GK Góbé	A	301	3.74	0.74
	A+O	313	3.98	1.26
GK Élet	A	397	4.14	0.88
	A+O	475	2.53	0.84
Overall	A	5642	4.04	1.65
	A+O	8884	3.33	1.15

Table 2
Mean separations of wheat genotypes for callus induction and plant regeneration

Genotype	No. of anthers cultured	Transformed mean values*	
		Callus induction	Regeneration
Pioneer 2550	3960	0.230a	0.122a
Cardinal	1880	0.119b	0.060ab
Caldwell	4968	0.051c	0.024b
GK Délibáb	2232	0.182ab	0.106a
GK Góbé	614	0.145ab	0.057ab
GK Élet	872	0.155ab	0.054ab

*Means followed by the same letters are not significantly different.

Data on 33 randomly selected Petri dishes showed that the frequency of albino plants was high without (64%) as well as with (68%) ovules. These data were based on 80 and 33 plants, respectively. Even though these frequencies are within the range found in wheat (Zhou and Konzak, 1989; Ziegler et al., 1990) and many factors affect albinism in anther culture (Ouyang et al., 1983; Hassawi and Liang, 1990; Navarro-Alvarez et al., 1994; Jahne and Lorz, 1995; Loschenberger et al., 1995), co-culture did not reduce albinism.

In wheat × wheatgrass backcrosses, anther callus induction increased with an increase in the backcross generations as well as with the co-culture of ovules (Table 3). Thus, the co-culture of ovules with anthers appeared to be useful in wide crosses. It may be pointed out that statistical testing of this data was not possible due to lack of proper design. The increase in callus induction with an increase in backcross generations was possibly due to improvement in fertility (Sharma, 1995).

Table 3
Percentage of anthers that callused (and number of anthers cultured) without and with ovaries from their florets in wheat × wheatgrass backcrosses

Cross	Generation	% callus induction	
		Without ovary	With ovary
Wheat × *Th. elongatum*	BC_1	0.00 (227)	0.35 (245)
Wheat × *Th. intermedium*	BC_2	0.00 (197)	1.26 (201)
	BC_3	1.81 (1081)	4.97 (1083)
Wheat × *Th. trichophorum*	BC_2	1.03 (309)	1.34 (327)
	BC_3	2.34 (1613)	4.05 (1534)
Average		1.65 (3427)	2.95 (3390)

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VARIABILITY AND INTERRELATIONSHIPS BETWEEN TRAITS OF TWO MAIZE POPULATIONS

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Synthetic maize populations are used in breeding as a source of superior inbred lines to be used as parental components of hybrids. Estimates of genetic variability, broad-sense heritability, and genetic and phenotypic correlations for grain yield, delayed silking, percentage of barren plants, grain moisture content and number of ears per plant were studied in two broad-base synthetic maize (*Zea mays* L.) populations, NS796A/92 and NS2040B/92. The nested design (random model) method was applied to assess the performances of half-sib families under conditions of severe drought during the 1994 and 1995 growing seasons in two locations. No significant differences were found between the populations for grain yield, delayed silking and number of ears per plant, but population NS796A/92 had a significantly lower percentage of barren plants and grain moisture content than population NS2040B/92. Broad-sense heritability estimates were relatively high for grain yield (51.4% and 58.9%), percentage of barren plants (52.4% and 54.1%), delayed silking (47.1% and 37.3%), grain moisture (60.4% and 60.8%) and number of ears per plant (58.9% and 66.7%). A highly significant positive genetic correlation was found between grain yield and grain moisture in population NS796A/92 (r_g=0.77**) and between grain yield and number of ears per plant in population NS2040B/92 (r_g=0.99**). Grain yield was negatively correlated with the percentage of barren plants and delayed silking in both populations.

Key words: maize populations, variability, correlation, half-sib recurrent selection

Introduction

Jenkins (1940) suggested recurrent selection as a long-term breeding procedure in maize breeding. This method was based on half-sib family selection, that emphasised selection for additive genetic effects. Assuming that superdominance has the greatest importance in yield inheritance, Hull (1945) suggested a recurrent selection scheme for the improvement of specific combining abilities. The HS-RS method was efficient in selection for grain yield in maize populations (Lamkey, 1992). After five cycles of HS-RS the combining abilities of Kolkmeier and Lancaster were improved, while the grain yield was either at the level of the initial population (Kolkmeier) or decreased (Lancaster) (Walejko and Russell, 1977). Coors (1988) found a 3.5% increase per selection cycle, while no significant changes in combining abilities occurred in maize populations with a narrow genetic basis (A635, W182E and W64A). The modified method of reciprocal recurrent selection using inbred testers did not increase the yield of the BS21 and BS22 populations (Russell et al., 1973).

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Greater maize yields are achieved in years with an above-average amount of rainfall, whereas rainfall deficits, especially at pollination, cause yield losses. Because the period of pollination is critical for maize development, an increasing amount of attention is being devoted to the study of pollination mechanisms (pollination and silking) under stress conditions (Herrero and Johnson, 1981; Guei and Wassom, 1992; Bolanos and Edmeades, 1993).

The objectives of the present study were to test the variability and relationships of some agronomic characters of two maize synthetic populations grown under drought conditions, and to select the best S_1 progenies and recombine them for the next cycle of RS on the basis of the characteristics of HS families.

Materials and methods

Two maize synthetics were selected having a broad genetic base: NS796A/92 and NS2040B/92. Both contained germplasm originating from the local maize population, Vukovarski Zuti Zuban, as well as some germplasm originating from the Iowa Stiff-Stalk Synthetic (BSSS). Vukovarski Zuti Zuban is an open-pollinated local population well-adapted to semi-arid growing conditions and was widely grown in the Yugoslav corn belt before the introduction of maize hybrids in the mid-20th century (Gibsman, 1956). It has been used to develop numerous inbreds, among which NS796/II and NS298/II are two of the best. Inbred line NS796/II has a shorter, cone-shaped ear, each bearing 14 to 16 grain rows, while NS298/II has a longer ear with 12 to 14 grain rows and a higher 1000-grain mass (Savic, 1968). Line NS298/II matures 5 to 7 days earlier than NS796/II. Both lines combine well with Lancaster Sure Crop-type lines, have very good adaptability, and are susceptible to the causal agent of stalk and root rot (*Fusarium graminearum*). The Iowa Stiff Stalk Synthetic (BSSS) was developed by Sprague (1946).

Synthetic population NS796A/92 was developed by crossing NS796/II with public lines containing germplasm from BSSS (B73, A632, CM105, H100 and B68). After testing a total of 218 inbred lines from the S_2 to S_5 generation at three locations (Srbobran, Bajmok and Uljma) in 1991, 13 were selected. Initial population NS796A/92 was developed after the recombination of these 13 selected lines.

The second population, NS2040B/92, was developed by crossing recently developed elite inbred lines of BSSS origin with a local inbred line. Line NS1141, one of the newest versions of line B73, was used as a recurrent parent in crosses with NS911 and NS568 (B73 derivations), NS298 (local plasma) and CM105 (public line). Fifteen of 220 lines from the S_2 and S_3 generations were selected after evaluation at three locations (Srbobran, Bajmok, Uljma) in 1991, and their plant to plant recombination in a winter nursery gave the population NS2040B/92. A cyclical improvement of both populations will be undertaken using the recurrent selection procedure in advanced generations.

In 1992, 300 plants per population were grown, and approximately 200 plants were selfed. The S_1 families were crossed with the unrelated inbred tester Mo17-46 in 1993. Based on the amount of seeds of HS families and the resistance of S_1 families to leaf, stem and ear diseases (*Helminthosporium turcicum* and *Fusarium graminearum*), 50 HS families were selected from each population.

In 1994 and 1995 field trials were established (with the selected HS families) according to the method of nested design - random model (Cochran and Cox, 1957) at two locations (Novi Sad and Srbobran). Each population was divided into two sets with 25 HS families per set, three replications within a set, and 40 plants per replication. The trials were established on a chernozem soil type, and the sowing was done by hand, with two seeds per hill. The amount of mineral fertilizers was determined according to soil provision with N, P, and K. Data were collected for grain yield (t ha^{-1}adjusted to 14% grain moisture), delayed silking, number of ears per plant, percentage of barren plants, and percentage grain moisture at harvesting. The significance of differences between the population means was tested using Model I ANOVA according to Sokal and Rohlf (1981).

Results

The mean square values for grain yield (GY), barren plants (BP), delayed silking (DS), grain moisture content (GM) and number of ears per plant (E/P) for the families within sets for both maize synthetics can be seen in Table 1. Differences among the locations, family/sets, and family × locations/sets were the only significant source of variation for all the traits in both populations. The null hypothesis on the equality of two means from two samples was accepted for grain yield, barren plant percentage and number of ears per plant, since the calculated F values were lower than the expected values. The null hypothesis was rejected for delayed silking and grain moisture percentage, because the calculated F values were higher than the expected F values. Population NS796A/92 and NS2040B/92 differed only with regard to barren plants and grain moisture percentage (Table 2).

The phenotypic variances for all traits in both populations were higher than the genetic variances. Genetic variances were considered to be significant if they were at least double the values of the corresponding standard errors (Falconer, 1981). The calculated values for genetic variance for HS families were significant for all traits in both populations except for delayed silking in population NS2040B/92. Higher genetic variances of all traits, except delayed silking and ears per plant, were found in population NS2040B/92 than in NS796A/92 (Table 3).

The highest heritability estimate was for grain moisture (60.4%) in population NS796A/92 and for number of ears per plant (66.7%) in population NS2040B/92. The lowest heritability estimate in both populations was for delayed silking (47.1% and 37.3%, respectively) which suggests the strong influence of environmental conditions on the expression of this trait. A comparison of the level of genetic variances of different traits is not possible because they are expressed in different units of measurement and exhibit a broad range of mean values. In such cases, estimates of the coefficient of genetic variation ensure reliable comparison. The highest coefficient of genetic variation in both populations was for the percentage of barren plants (CV_g = 56.5% and CV_g = 50.2%). The lowest CV_g values were for the number of ears per plant (CV_g = 1.8%) in population NS796A/92 and for grain moisture (CV_g = 3.6%) in population NS2040B/92. The coefficients of genetic variation for grain yield were CV_g = 6.1% for population NS796A/92 and CV_g = 10.9% for NS2040B/92.

Population NS796A/92 exhibited negative functional dependence between grain yield and the percentage of barren plants (r_g = –0.99**, r_p = –0.70**) as well as a weak negative correlation between delayed silking and grain yield (r_g = –0.34* and r_p = –0.10) (Table 4). Grain moisture showed a significant positive correlation number of ears per plant a non-significant positive correlation with grain yield. The correlations between the percentage of barren plants and the number of ears per plant (r_g = –0.59,** r_p = –0.56**) were

significantly negative. The correlations between delayed silking and number of ears per plant were of opposite sign (r_g = –0.59**, r_p = 0.24). Similar relationships were found in the population NS2040B/92 between grain yield and number of ears per plant (r_g = 0.99**, r_p = 0.82**), between grain yield and percentage of barren plants (r_g = –0.99**, r_p = –0.77**) and between the percentage of barren plants and number of ears per plant (r_g = –0.99**, r_p = –0.97**) (Table 4).

Table 1

Mean square values for grain yield (GY), barren plants (BP), delayed silking (DS), grain moisture (GM) and number of ears per plant (E/P) from the analysis of variance of two sets of half-sib families

Source of variation	Populations	df	GY	BP	DS	GM	E/P
Locations	NS796A/92	3	236.97**	1108.24**	46.08**	19.38**	1.00^{-2}**
	NS2040B/92		542.51**	1408.13**	32.69**	411.91**	1.16^{-2}**
Set	NS796A/92	1	0.07	24.73**	0.18*	0.14	4.00^{-3}
	NS2040B/92		13.28**	27.39**	1.64**	1.15**	7.00^{-3}
Loc x Set	NS796A/92	3	0.54**	125.74**	0.50	5.55**	1.00^{-3}*
	NS2040B/92		7.18**	139.01**	0.40	2.15**	9.00^{-3}*
Reps/Set/Loc	NS796A/92	8	0.13	58.68	0.49	0.88**	1.00^{-3}**
	NS2040B/92		1.65**	57.91	0.43	2.56**	3.00^{-3}
Fam/Set	NS796A/92	48	1.47**	117.98**	2.05**	2.14**	2.23^{-3}**
	NS2040B/92		3.69**	145.06**	2.35**	6.32**	1.60^{-2}**
Fam × Loc/Set	NS796A/92	144	0.70**	56.18**	1.10**	0.86**	9.20^{-4}**
	NS2040B/92		1.16**	66.58**	1.45**	2.49**	5.00^{-3}**
Error	NS796A/92	192	0.52	37.08	0.27	0.04	6.90^{-4}
	NS2040B/92		0.45	38.98	0.36	0.15	3.00^{-3}

*;** Significant at the 5% and 1% levels

Table 2

Mean values of five traits of two synthetic maize populations

Population	Grain yield (t ha^{-1}) (GY)	Barren plants (%) (BP)	Delayed silking (DS)	Grain moisture (%) (GM)	Ears per plant (E/P)
NS 796 A/92	7.20	2.04	3.10	25.32	0.99
NS 2040 B/92	7.31	4.41	3.02	27.53	0.96
F observed	0.46	6.93	0.20	4.01	2.69
F critical $_{0.05(1.98)}$			3.94		
F critical $_{0.01(1.98)}$			6.90		

Table 3
Estimates of variance components, heritability, and coefficients of variation of HS families for NS 796 A/92 and NS 2040B/92 populations

Traits[1]	Populations	Parameters						
		V_g	SE_{Vg}	V_p	H^2 (%)	SE_{h^2}	CV_g(%)	CV_p(%)
GY	NS796A/92	0.19	0.08	0.37	51.35	0.22	6.06	8.41
	NS2040B/92	0.63	0.19	1.07	58.87	0.21	10.90	13.15
BP	NS796A/92	15.45	6.53	29.49	52.38	0.22	56.48	78.04
	NS2040B/92	19.62	7.98	36.26	54.10	0.22	50.18	68.22
DS	NS796A/92	0.24	0.12	0.51	47.06	0.23	15.69	23.08
	NS2040B/92	0.22	0.14	0.59	37.29	0.23	15.68	25.34
GM	NS796A/92	0.32	0.11	0.53	60.38	0.21	2.24	2.89
	NS2040B/92	0.96	0.34	1.58	60.76	0.21	3.55	4.57
E/P	NS796A/92	0.001	0.00	0.00	58.88	0.22	1.83	2.39
	NS2040B/92	0.001	0.00	0.00	66.71	0.21	5.35	6.55

[1]V_g and V_p are estimates of genetic and phenotypic variances, respectively; H is the estimate of heritability; CV_g and CV_p are estimates of genetic and phenotypic coefficients of variation, respectively; and SE is the standard error. GY: grain yield, BP: barren plants, DS: delayed silking, GM: grain moisture, E/P: ears per plant

Table 4
Genetic (r_g) (above) and phenotypic (r_p) correlations (below diagonal) between studied traits in the populations NS 796 A/92 and NS 2040B/92

Traits[1]	Populations	GY	BP	DS	GM	E/P
GY	NS796A/92		–0.999**	–0.343**	0.773**	0.049
	NS2040B/92		–0.999**	–0.247	0.055	0.999**
BP	NS796A/92	–0.695**		0.249	–0.062	–0.586**
	NS2040B/92	–0.774**		0.192	0.313*	–0.999**
DS	NS796A/92	–0.101	0.087		0.289*	–0.593**
	NS2040B/92	–0.108	0.661**		0.143	–0.321*
GM	NS796A/92	0.431*	–0.033	0.095		–0.002
	NS2040B/92	0.013	0.253	0.320*		–0.327*
E/P	NS796A/92	0.239	–0.561**	–0.237	–0.009	
	NS2040B/92	0.824**	–0.971**	–0.338*	–0.311*	

*,** Significant at the 5% and 1% levels; [1]GY: grain yield, BP: barren plants, DS: delayed silking, GM: grain moisture, E/P: ears per plant

Discussion

Although populations of different origin were studied (local plasma originating from Vukovarski Zuti Zuban was dominant in population NS796A/92, while BSSS germplasm was dominant in NS2040B/92), there were no clear differences between them for many traits. The masking effect of the dominant alleles of elite inbred testers and the interaction between the dominant alleles of the population and the testers may be the reason for the lack of a clear

difference between the traits of the two populations. All HS families have 50% of the germplasm from the common parent, which reduces the variability among the families. Hence, the inbred families are more suitable for the evaluation of the genetic variability of the populations than the test crosses. Ivanovic et al. (1987) studied the correlations for grain yield between inbred (S), full-sib (FS), and half-sib (HS) progenies in populations having a narrow (NBP) and a broad (BBP) genetic basis. Variability for grain yield among the inbred progenies was greater than among the HS families.

Heritability depends largely on population and environment conditions, and the interpretation of the heritability values from different experiments should be made carefully (Lamkey and Hallauer, 1987). According to 121 experiments on the progenies from seven programmes of recurrent selection, the heritability for grain yield ranged from 53.3 to 79.8%, while Johnson (1989) found lower heritability for grain yield (15.2%) in 285 test-cross families. In the present study, broad sense heritability for grain yield and many other traits was relatively high. The high heritability of the examined traits may be explained by the inability of the nested design to separate the additive from the total genetic variance and by the lower phenotypic variance of the HS progenies in relation to the phenotypic variance of individual plants.

The increased number of barren plants caused reduced grain yield. Under favourable conditions for maize growing, the appearance and development of the silk is not a limiting factor for pollination and grain formation. However, stress conditions caused by drought or high plant density result in delayed silking (Herrero and Johnson, 1981; Hall et al., 1981). Studying eight open-pollinated populations, seven synthetic populations and five composites, Martinello and Lorenzoni (1985) found a negative correlation between delayed silking and grain yield ($r = -0.78$). In the present study, there was also a negative correlation between grain yield and delayed silking ($r_g = -0.34^*$, $r_g = -0.25$), albeit a weaker one than in the above study.

The expression of more ears per plant may increase the yield potential. Direct selection for grain yield often brings about negative correlation responses for other traits, such as plant height, height up to the base of the upper ear, and grain moisture. Reddy et al. (1990) found that selection for more ears per plant increased the grain yield with no negative effects on other agronomically important traits, so that it may be used as a suitable criterion in selection for grain yield. Souza et al. (1985) found a high genetic correlation between prolific plants and grain yield ($r_g = 0.94$). In the present study, the genetic correlation between grain yield and prolific plants was positive, but it was significant only in population NS2040B/92 ($r_g = 0.99^{**}$). In fact, an increased number of ears per plant brings about a reduced percentage of barren plants, a shorter interval between pollination and silking, and a slight reduction of grain moisture in both populations.

The lower percentage of barren plants and more ears per plant in the population NS796A/92 relative to the population NS2040B/92 indicated that the former has better adaptability to local conditions. Additionally, NS796A/92 has

shorter vegetative growth, so that the critical phase of development (pollination) is finished before the period of severe drought (July and August). With the aim of increasing the frequency of favourable alleles for traits that affect the grain yield under drought conditions (percentage of barren plants, number of ears per plant, delayed silking), several cycles of HS recurrent selection should be performed in combination with inbred progeny selection in the population NS796A/92. On the other hand, direct selection for grain yield would be more efficient in the population NS2040B/92, as this population has a higher average yield and higher genetic variance for the same traits as in the NS796A/92 population.

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CHARACTER ASSOCIATION AND PATH ANALYSIS OF YIELD AND ITS COMPONENTS IN HOT PEPPER (*CAPSICUM ANNUUM* L.)*

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Eighteen hot pepper genotypes were studied at Melkasa Research Center in a randomized complete block design with three replications to determine the relationships among the major characters and their direct and indirect effects on fruit yield. Fruit yield per plant had positive and significant correlations with canopy width, plant height, leaf area and fruit number per plant. Some characters also showed significant positive correlations among themselves. Path coefficient analysis revealed that canopy width, leaf area, fruit number per plant and pericarp thickness had positive direct effects on the fruit yield per plant. Therefore, selection based on these characters will lead to an increase in fruit yield per plant in hot pepper.

Key words: breeding, correlation, genotypic, path, phenotypic

Introduction

Hot pepper (*Capsicum annuum* L.) is one of the most important cash crops which is used as vegetable, spices, condiment, pickles and sauces in Ethiopia. In addition to its extensive domestic consumption, the crop is used as a raw material for local industries for oleoresin extraction for the export market. In view of the importance of this crop and the low yields currently achieved, an improvement programme has been started at the Ethiopian Agricultural Research Organization (the former Institute of Agricultural Research) to develop high-yielding varieties.

Yield is a complex polygenic character that depends directly or indirectly on a number of traits. Correlation coefficients are indicative of the extent of association between yield and its components. Though the estimation of simple correlation coefficients helps the breeder to determine the relationships between yield and other components, it does not show the direct and indirect contributions of each character towards yield (Bhatt, 1973). The identification of direct contributing characters is essential for improving breeding efficiency (Ebong, 1972). The partitioning of the correlation coefficients and the assessment of the relative importance of each causal factor affecting yield are possible only through path coefficient analysis.

Path coefficient analysis for yield and its components by previous researchers showed that the number of fruits per plant had high, positive direct effects on the fruit yield (Gupta and Yadav, 1984; Lakashmaiah and Murty, 1984; Ahmed et al., 1997). Such work has never been undertaken on hot pepper

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under Ethiopian conditions. Hence, the objective of the present study was to identify the principal yield components that could be used in the improvement of the hot pepper yield through a planned breeding programme.

Materials and methods

Eighteen hot pepper genotypes were grown in a randomized complete block design with 3 replications during the rainy season of 1997/98 at Melkasa Agricultural Research Center. Fifty-day-old seedlings were transplanted at a spacing of 70 cm between rows and 30 cm between plants in the row. Observations on days to flowering, plant height, canopy width, leaf area, number of fruits per plant, fruit length, pericarp thickness, fruit width, mean fruit weight, 1000-seed weight and dry fruit yield were recorded on five randomly selected plants from the central rows.

The phenotypic and genotypic path coefficients were determined by the method of Dewey and Lu (1959).

Results and discussion

Estimates of phenotypic and genotypic correlations are given in Table 1. The genotypic correlation coefficients were higher in magnitude than the corresponding phenotypic ones, indicating the inherent associations between various characters. However, both phenotypic and genotypic correlations were close to each other in each instance. This may have been due to the great care taken during data collection, which tended to reduce the error in environmental variance to minor proportions. If the environmental variance was reduced to zero, the phenotypic and genotypic correlations would have been identical, as was pointed out by Dewey and Lu (1959). Since the two types of correlations were similar, references will be made only to genotypic correlations to avoid repetition.

Table 1

Estimates of phenotypic (upper right diagonal) and genotypic (lower left diagonal) correlations between fruit yield per plant and other characters

Characters*	DFr	PlHt	CW	LA	FtN	FtL	PcTh	FtW	MFtWt	SWt	FY
DFr	1	0.391	0.299	0.346	0.304	–0.099	–0.001	0.017	–0.208	0.020	0.134
PlHt	0.561	1	0.444	0.612**	0.383	0.135	0.117	0.129	–0.036	–0.042	0.596**
CW	0.404	0.560	1	0.337	0.434	0.216	–0.030	–0.311	–0.309	–0.222	0.655**
LA	0.468	0.777	0.539	1	0.324	0.090	–0.037	0.003	–0.124	–0.247	0.491*
FtN	0.423	0.438	0.574	0.343	1	–0.111	–0.277	–0.551*	–0.556*	–0.251	0.492*
FtL	–0.178	0.221	0.419	0.322	–0.136	1	0.293	–0.516*	0.187	–0.019	0.288
PcTh	0.058	0.165	–0.061	–0.037	–0.381	0.535	1	0.516*	0.646**	0.485*	0.178
FtW	0.046	0.152	–0.392	–0.146	–0.571	0.003	0.716	1	0.758**	0.504*	–0.126
MFtWt	–0.226	–0.083	–0.314	–0.162	–0.633	0.361	0.855	0.756	1	0.584*	0.028
SWt	0.003	0.106	–0.436	–0.329	–0.394	0.006	0.805	0.679	0.729	1	–0.124
FY	0.305	0.695	0.948	0.681	0.501	0.640	0.276	–0.205	–0.042	–0.156	1

With 16 degrees of freedom values greater that 0.468 and 0.590 are significant at the 0.05 and 0.01 probability levels, respectively; *, ** - indicate significant differences at the 5 and 1% levels, respectively; * DFr = days to flowering, PlHt = plant height, CW = canopy width, LA = leaf area, FtN = fruit number per plant, FtL = fruit length, PcTh = pericarp thickness, FtW = fruit width, MFtWt = mean fruit weight, SWt = 1000 seed weight, FY = fruit yield

Canopy width, plant height, leaf area, fruit length and fruit number per plant showed positive and significant correlations with fruit yield. Significant positive correlations were also observed between some of the characters. Plant height was significantly correlated with canopy width and leaf area; canopy width with leaf area and fruit number per plant; pericarp thickness with fruit width and mean fruit weight; mean fruit weight with pericarp thickness and fruit width; and fruit width with pericarp thickness and 1000 seed weight. However, fruit width was negatively and significantly correlated with fruit number and mean fruit weight. The positive significant correlation of leaf area with both plant height and fruit yield may have been due to larger plants having a larger leaf surface, photosynthesising higher quantities of carbohydrates that were directed to fruit formation.

In general, many of the characters were positively or negatively correlated due to mutual association with other characters. The results of path coefficient analysis to separate out the direct and indirect causes of association are presented in Table 2 and Figure 1.

Fruit yield vs. canopy width

The total correlation between fruit yield and canopy width was positive and significant ($r=0.948$, $P<0.01$). The direct effect of canopy width on fruit yield was also very high (1.110) and positive. In addition canopy width registered moderately high indirect and positive effects on fruit yield via leaf area (0.463) and mean fruit weight (0.229). The negative indirect influences of canopy width were registered through days to flowering, fruit length and plant height. The result indicated that canopy width was an important yield component. Similar views were expressed by Gupta and Yadav (1984) and Ahmed et al. (1997).

Table 2

Direct (underlined) and indirect genotypic paths of fruit yield and its components

Chrts*	DFr	PlHt	CW	LA	FtN	FtL	PcTh	FtW	MFtWt	SWt	r_g*
DFr	–0.682	–0.218	0.448	0.402	0.014	0.101	0.076	–0.002	0.165	0.001	0.305
PlHt	–0.382	–0.389	0.622	0.667	0.014	–0.126	0.216	–0.006	0.061	0.019	0.695
CW	–0.275	–0.217	1.110	0.463	0.018	–0.238	–0.080	0.017	0.229	–0.078	0.948
LA	–0.319	–0.301	0.598	0.858	0.011	–0.183	–0.048	0.006	0.118	–0.059	0.681
FtN	–0.288	–0.170	0.637	0.294	0.013	0.077	–0.498	0.024	0.462	–0.071	0.501
FtL	0.121	–0.086	0.465	0.276	–0.004	–0.569	0.699	0.000	–0.264	0.001	0.640
PcTh	–0.040	–0.064	–0.068	–0.031	–0.012	–0.304	1.307	–0.030	–0.625	0.144	0.276
FtW	–0.031	–0.059	–0.435	–0.125	–0.018	0.002	0.935	–0.042	–0.552	0.121	–0.205
MFtWt	0.154	0.032	–0.348	–0.139	–0.020	–0.205	1.112	–0.032	–0.730	0.131	–0.042
SWt	–0.002	–0.041	–0.484	–0.282	–0.012	–0.003	1.052	–0.028	–0.532	0.179	–0.156

* DFr = days to flowering, PlHt = plant height, CW = canopy width, LA = leaf area, FtN = fruit number per plant, FtL = fruit length, PcTh = pericarp thickness, FtW = fruit width, MFtWt = mean fruit weight, SWt = 1000 seed weight; *r_g = genotypic correlation coefficients; $P_{RY}=\sqrt{(-0.173)}$

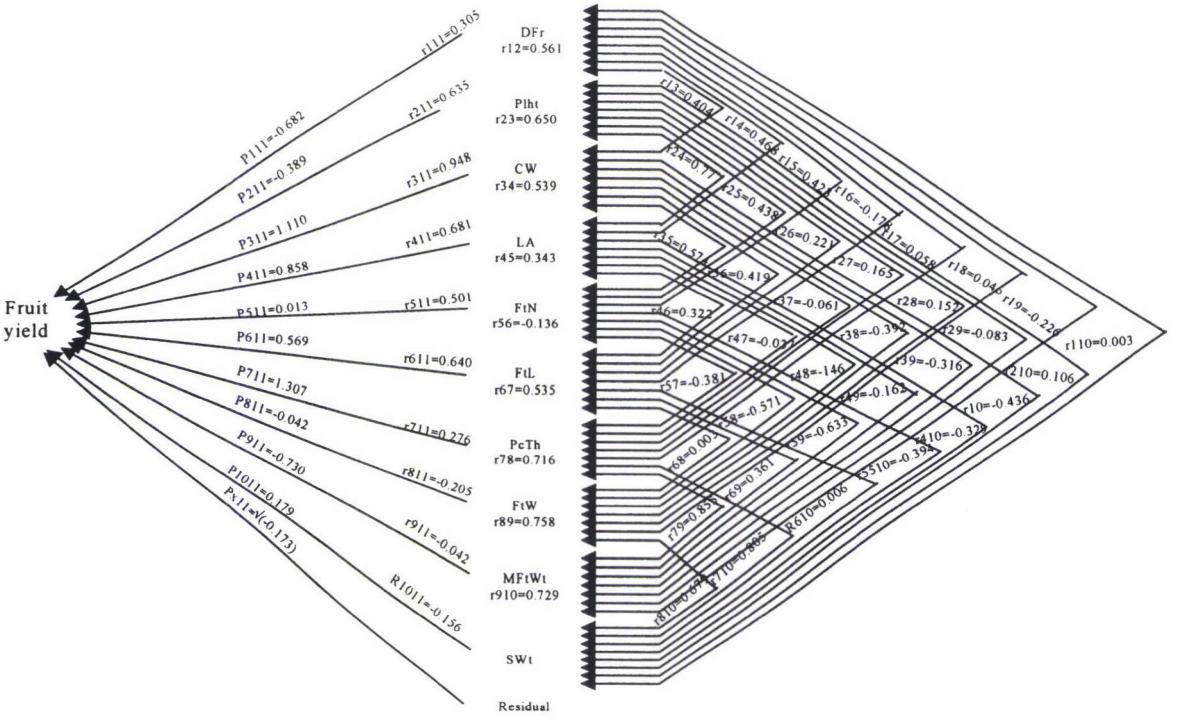

Fig. 1. Path diagram of yield and its components in hot pepper. One-sided arrows show path coefficients, two-sided arrows show genotypic correlation coefficients

Fruit yield vs. leaf area

The correlation between fruit yield and leaf area was positive and significant ($r=0.681$, $P<0.01$). The direct effect of leaf area on fruit yield was also high (0.858). A positive indirect influence of leaf area on fruit yield was recorded via canopy width (0.598). Negative indirect influences of leaf area on fruit yield were recorded via days to flowering and plant height. The high, positive direct influence of leaf area on fruit yield indicated that the character was an important yield component.

Fruit yield vs. pericarp thickness

The correlation between fruit yield and pericarp thickness was small but positive. The direct effect of pericarp thickness on yield was very high and positive (1.307). The low correlation value was due to the high negative indirect effect of pericarp thickness on fruit yield through mean fruit weight (–0.625) and fruit length (–0.304), which counterbalanced the high direct effect. Under these circumstances, restrictions should be imposed to nullify the undesirable indirect effects in order to make use of the direct effect.

Yield vs. plant height

The correlation between fruit yield and plant height was positive and significant ($r=0.695$). The direct effect of plant height on fruit yield was negative (–0.389), as reported by Depestre and Gomez (1993). The indirect influences of plant height on fruit yield via canopy width (0.622) and leaf area (0.667) were positive and high. These two large positive indirect effects made the overall correlation between plant height and fruit yield high and positive. The result indicated that the two factors should be considered simultaneously during the application of direct selection through plant height.

Fruit yield vs. fruit number

The total correlation between fruit yield and fruit number was high, positive and significant ($r=0.501$, $P<0.05$) as reported by Ahmed et al. (1997). This was the result of the high, positive indirect influences of fruit number per plant through canopy width (0.637), mean fruit weight (0.462) and leaf area (0.294). On the other hand, the direct influence of fruit number per plant on fruit yield was low (0.013). The result revealed that during selection for high yield through fruit number per plant, considerations should be given to canopy width, mean fruit weight and leaf area.

Fruit yield vs. fruit length

The total correlation between fruit yield and fruit length was positive and significant ($r=0.640$, $P<0.01$). However, the direct influence of fruit number per plant on fruit yield was negative and high (–0.569). The strong correlation between fruit yield and fruit length was due to the high, positive indirect influences of fruit length on fruit yield through pericarp thickness, canopy width

and leaf area (0.699, 0.465 and 0.276, respectively). The results showed that fruit length was not an important yield component.

The residuals were negative and non-significant, indicating that there was no need to include more yield components in the path coefficient analysis.

Conclusion

Significant ($P<0.05$) positive correlations were found with plant height, canopy width, leaf area, fruit number per plant and fruit length. These findings tentatively suggested that these characters were the primary components of fruit yield in hot pepper. Path coefficient analysis, however, showed that canopy width, leaf area and pericarp thickness were the main yield components having maximum direct effects on fruit yield, as previously reported by Gupta and Yadav (1984), Depestre and Gomez (1993) and Ahmed et al. (1997). Therefore, selection for a combination of these characters would be effective in increasing the fruit yield in hot pepper. Since fruit yield was low in taller plants, short-statured plant idiotypes having a wider canopy width and larger leaf area would probably give the best fruit yields.

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SAPROBIC FUNGI INHABITING TOMATO PHYLLOPLANE AS POSSIBLE ANTAGONISTS OF *ALTERNARIA SOLANI*

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The effect of saprobic fungi inhabiting the tomato phylloplane on the expression of tomato early blight (caused by *Alternaria solani*) was evaluated.

Artifical inoculation with the pathogen and the antagonists was carried out in a commercial greenhouse on four-month-old tomato plants (cv. Parador, Asgrow Seeds, Co.). Inoculation with the antagonist was made an hour before inoculation with the pathogen.

Inoculum of saprobic fungi was produced on potato-dextrose agar (PDA). The concentration of the microorganism propagules was diluted to 10^7 c.f.u./ml. *Alternaria solani* inoculum was obtained on cornmeal agar and the concentration used for the inoculation was 10^5 c.f.u./ml.

A disease scale was established and the percentage of infection was assessed at 21, 44 and 86 days from the beginning of the assay. A disease progress curve was fitted to the values and the area under the disease progress curve (AUDPC) and the epidemic rate were calculated. From the statistical analysis of these parameters it could be concluded that *Nigrospora* spp., *Penicillium* sp. b, *Chaetomium globosum, Cladosporium cladosporioides* and *Trichoderma polysporum* might be potential biological control agents of *Alternaria solani.*

Although the practical application of this method is difficult, it may provide several potential benefits in the reduction of pollution and the enhancement of the biological balance stability.

Key words: *Alternaria solani*, biological control, antagonists, phylloplane

Introduction

The protected cropping of tomato (*Licopersicum esculentum* Mill) is one of the most typical activities in the green belt surrounding La Plata city (Buenos Aires Province, Argentina). The environmental conditions characteristic of protected crops promote the development of a number of foliar diseases. Early blight, caused by *Alternaria solani*, is one of the most important among them. This disease is controlled by spraying with protective fungicides. Fungicide applications are characterised by high frequency and high dosage.

The development of effective, economical and environmentally safe disease management methods represents a constant challenge for phytopathologists. The development of fungicide tolerance in pathogen populations (Delp, 1988) and economic pressures to reduce crop protection with

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pesticides as a whole have motivated major changes, especially reductions in fungicide treatments (Sutton, 1990).

To avoid the presence of fungicide residues and perform productivity, alternative, more effective methods for managing early blight are needed. The introduction of microorganisms as biological control agents is one possible option to fungicides in the management of disease caused by *Alternaria solani* (Brame and Flood, 1983; Kumar and Singh, 1983; Flood and Rees, 1986; Okasha et al., 1989; Sharma et al., 1988; Niwas and Sharma, 1988).

Current research focuses on antagonist microorganisms inhabiting the leaf surface or phylloplane. Some of these agents exhibit antibiosis to *Alternaria solani in vitro* (Niwas and Sharma, 1988; Kumar and Singh, 1983). The results of many *in vitro* assays also demonstrate that some phylloplane fungi may play an important role in reducing disease incidence in many *Alternaria* species (Brame and Flood, 1983; Flood and Rees, 1986; Blakeman, 1988; Okasha et al., 1989).

The objective of this research was to screen and select fungal antagonists which inhabit the tomato phylloplane under plastic greenhouses and to test them for the biological control of early blight caused by *Alternaria solani*.

Materials and methods

Inoculum production for potential biological control agents

Filamentous fungi and yeasts were isolated from tomato leaves grown in commercial greenhouses in the La Plata green belt (Mónaco, 1997). Inoculum of saprobic organisms was obtained by growing on potato-dextrose agar (PDA). Filamentous fungi were incubated in a growth chamber for 15 days (20°C, 12 hours light). Yeasts were cultured for 10 days at 25°C in darkness. Conidia of the antagonists were suspended in sterile distilled water plus surfactant (0.05 ml Triton X-100/100 ml), filtered through three layers of cheesecloth, counted with the aid of a hemocytometer, and diluted to the desired concentrations in water plus surfactant. The propagule concentration was diluted to 10^7 c.f.u./ml.

Alternaria solani inoculum production

Alternaria solani isolate A95 was obtained from tomato leaves that showed typical disease symptoms using the normal phytopathological methods.

Inoculum was produced on cornmeal agar. The cultures were illuminated with fluorescent light for 8 hours in order to promote sporulation (Zhu et al., 1985). A spore suspension was obtained in the same way as was described for the antagonists. Dilutions were made in order to reach a concentration of 10^5 c.f.u./ml.

Greenhouse biocontrol assays

The assay was carried out in a commercial greenhouse located in Echeverry, Buenos Aires Province, Argentina. A completely randomized block design with four repetitions was chosen for the experiment. The experimental units consisted of single plants. One bordered area (7 × 7 m) was located within the crop. The blocks were arranged in rows, and the two marginal rows bordering the remaining non-experimental area were kept as buffers. Tomato plants with 6 leaves (cv. Parador, Asgrow Seeds Co.) were inoculated four months after planting on November 30, 1995 at 28°C air temperature. Inoculum suspensions of the pathogen and the antagonist were poured into different sprayers. Two drops of commercial surfactant (Triton-X100) were added to each of the sprayers.

The upper portion of the plants was sprayed with antagonist inoculum until runoff. An hour later the *Alternaria solani* spore suspension was applied by spraying. Later, each of the plants was covered with transparent plastic film in order to form a moist chamber.

The treatments evaluated were:

1. Control I: plants sprayed with distilled water
2. Control II: plants sprayed with the pathogen (*Alternaria solani*)
3. Plants sprayed with *Penicillium* sp. a. Link + *A. solani*
4. Plants sprayed with *Alternaria alternata* (Fr.) Keissler + *A. solani*
5. Plants sprayed with *Fusarium semitectum* Berkeley and Ravenel + *A. solani*
6. Plants sprayed with *Chaetomium globosum* Kunze + *A. solani*
7. Plants sprayed with *Aspergillus niger* Van Tieghen + *A. solani*
8. Plants sprayed with *Cryptococcus luteolus* Kutzing + *A. solani*
9. Plants sprayed with *Trichoderma harzianum* Rifai + *A. solani*
10. Plants sprayed with *Penicillium* sp. b + *A. solani*
11. Plants sprayed with *Cladosporium cladosporioides* (Fresen) de Vries + *A. solani*
12. Plants sprayed with *Trichoderma polysporum* Rifai + *A. solani*
13. Plants sprayed with *Nigrospora* sp. Zimmermann + *A. solani*
14. Plants sprayed with *Epicoccum purpurascens* Ehrenberg + *A. solani*

Three records of disease severity were made to establish a disease progress curve (Kranz and Rotem, 1988). The first disease evaluation was made on 21/12/95, the second on 12/1/96 and the third on 31/1/96. For symptom evaluation all the leaflets with lesions from four leaves on the upper third of the plant were evaluated. The area under the disease progress curve (AUDPC) was calculated for each of the treatments following a trapezoidal integration method (Zadoks and Schein, 1979). To calculate the epidemic rate (r) the disease progress curves were linearized. In all the treatments linear transformation was carried out, with the EPIMODEL computer program.

The percentage of severity was calculated following Townsend and Heuberger (1943):

$$P = (n \times V) / Z \times N \times 100$$

where P: percentage of severity, n: number of leaflets with lesions in each category, V: value of maximum category, Z: total folioles analysed, N: total folioles analysed

Categories: 0: no disease
1: 0–25% severity
2: 26–50% severity
3: 50–75% severity
4: 76–100% severity

Qualitative rankings were established according to the scheme of Boff et al. (1996).

Statistical analysis

The data were analysed by ANOVA and mean separation was accomplished using the Tukey test; the test of significance was conduced at $P = 0.05$. Since the results of repeated experiments under similar conditions were similar, data from only one experiment are presented.

Results and discussion

Greenhouse biocontrol assays

The disease progress for the different treatments can be observed in Figure 1. From these data it can be concluded that, although plants formerly treated with *Cladosporium cladosporiodes*, *Penicillium sp. b, Trichoderma*

harzianum, Alternaria alternata and *Cryptococcus luteolus* all showed a significant reduction in initial disease compared to the control ($p<0,05$), only *Cryptococcus luteolus* and *Penicillium* sp. b resulted in substantially lower infection percentages. Since pathogen development was only inhibited when the antagonist had been previously inoculated, this suggests that the active growth of saprobic fungi on the leaf surface is an important factor in antagonistic ability. Statistical analysis shows that the AUDPC in plants inoculated with *Nigrospora* sp., *Penicillium* sp. b, *Chaetomium globosum, Cladosporium cladosporioides, Cryptococcus luteolus* and *Trichoderma polysporium,* differs significantly from the AUDPC recorded in plants inoculated exclusively with *Alternaria solani* (Fig. 2). These species are thus potential biological control agents of *Alternaria solani*, because AUDPC reduction is an important parameter of control, taking into consideration that this leads to a lower percentage of infection at harvest.

Brame and Flood (1983) obtained a significant reduction in the lesions provoked by *Alternaria solani* in tomato when the plants had been preinoculated with *Aureobasidium pullulans.*

The germination and active growth of *Cryptococcus luteolus* and *Penicillium* sp. b might have caused food source depletion on the leaf surface and a consequent reduction in the germination of *Alternaria solani* conidia, probably associated with a decrease in nutrient availability (Blakeman, 1988).

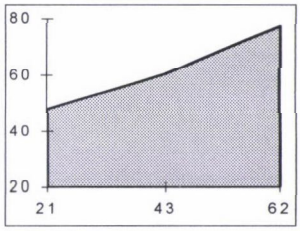
One of the antagonistic action mechanisms of *Trichoderma harzianum* is the release of fungitoxic metabolites (Dennis and Webster, 1971). This fact might explain the lower expression of initial disease when this fungus was inoculated. *Trichoderma harzianum* might have lost the ability to release this sort of metabolites by the end of the disease cycle. This might explain the absence of differences in the percentage of infection in this treatment compared to the control at the end of the cycle.

The results of disease progress curves are very useful because they allow epidemics to be considered as a dynamic, unidirectional system leading to an increase in disease (Zadoks and Schein, 1979).

One of the possible control measures would be a decrease in initial infection. This might be achieved by applying an antagonist in order to achieve a decrease in the infection percentage at the onset of epidemics. In this way lower AUDPC and disease level at harvest would be obtained. *Penicillium* sp. b, *Trichoderma polysporum*, *Cladosporium cladosporioides* and *Nigrospora* sp. might be suitable agents for this purpose. However, antagonists may exert their action by making disease progress slower, that is to say, by reducing the epidemic rate. *Cryptococcus luteolus* and *Chaetomium globosum* appear to be efficient in this kind of action (Fig. 3).

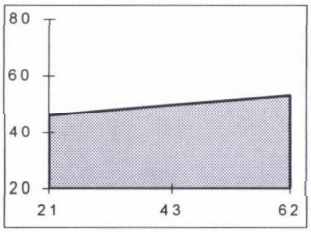
Filamentous fungi naturally inhabiting the leaf surface tend to become dominant at the beginning of the senescence period. This fact might make them less effective as direct biological control agents. Nevertheless, they can often behave as efficient antagonists. Such is the case for *Penicillium* sp. b, *Cladosporium cladosporioides, Trichoderma polysporum* and *Chaetomium globosum.*

A. solani



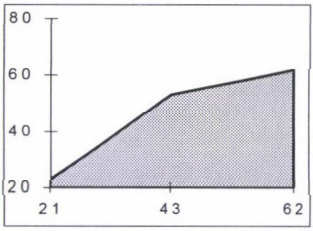
AUDPC=2499.75

Penicillium sp a vs A. solani



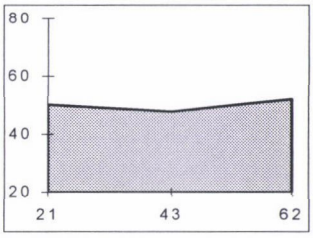
AUDPC=2036.4

A. alternata vs A. solani



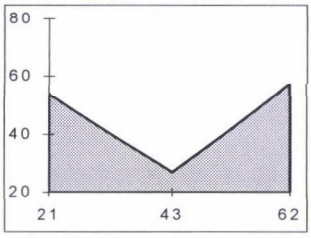
AUDPC=1934.65

F. semitectum vs A. solani



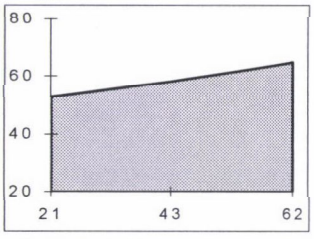
AUDPC=2022.8

Ch. globosum vs A. solani



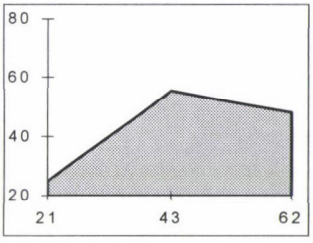
AUDPC=1691.85

A. niger vs A. solani



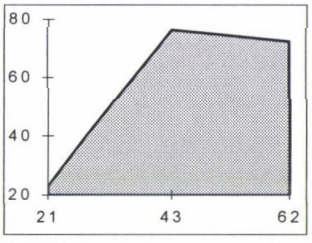
AUDPC=2397.55

C. luteolus vs A. solani



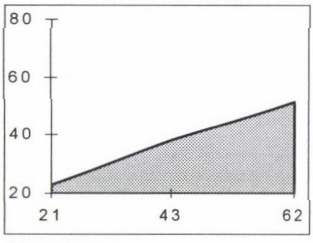
AUDPC=1870.65

T. harzianum a vs A. solani



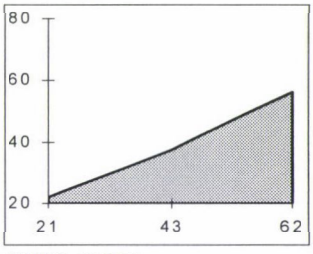
AUDPC=2497.85

Penicillium sp. b vs A. solani



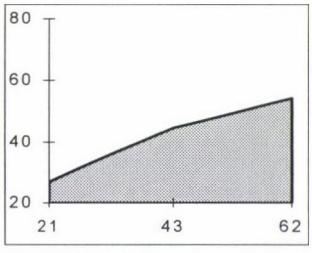
AUDPC=1519.35

Cl. cladosporioides vs A. solani



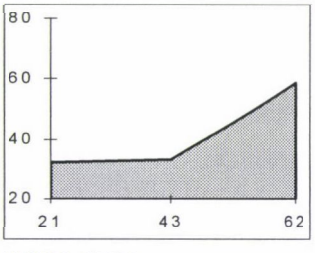
AUDPC=1547.5

T. polysporum vs A. solani



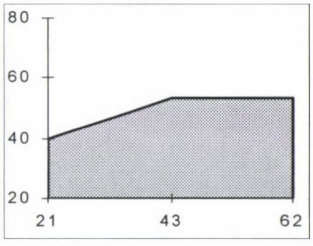
AUDPC=1731.25

Nigrospora sp. vs A. solani



AUDPC=1590.4

E. purpurascens vs A. solani



AUDPC=2039

Fig. 1. Area under the disease progress curve (AUDCP) in different treatments. Disease percentage produced by *Alternaria solani* in three evaluations (21, 43 and 62 days) and the AUDPC of control plants and those treated with the antagonists.

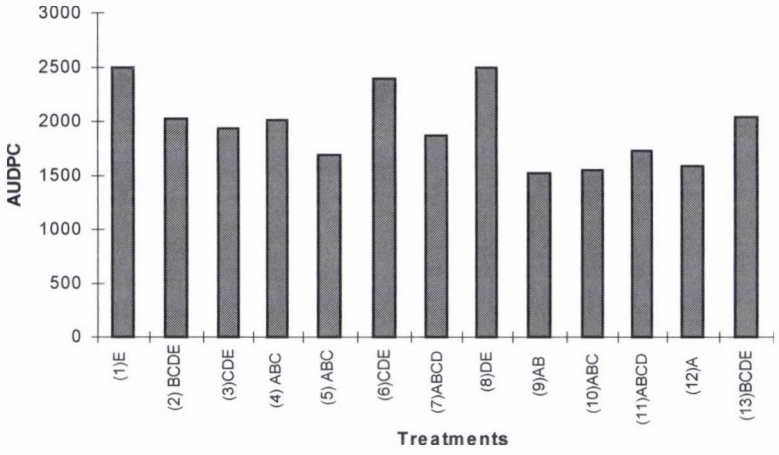


Fig. 2. Area under the disease progress curve (AUDPC) of *Alternaria solani* and the different treatments. 1. *Alternaria solani;* 2. *Penicillium* sp. a + *A. solani*; 3. *A. alternata* + *A. solani;* 4. *Fusarium semitectum* + *A. solani;* 5. *Chaetomium globosum* + *A. solani;* 6. *Aspergillus niger* + *A. solani;* 7. *Cryptococcus luteolus* + *A. solani;* 8. *Trichoderma harzianum* + *A. solani;* 9. *Penicillium* sp. b. + *A. solani;* 10. *Cladosporium cladosporioides* + *A. solani;* 11. *T. polysporum* + *A. solani;* 12. *Nigrospora* sp. + *A. solani;* 13. *Epicoccum purpurascens* + *A. solani*. Columns followed by the same letter do not differ significantly according to Tukey's test ($P<0.05$)

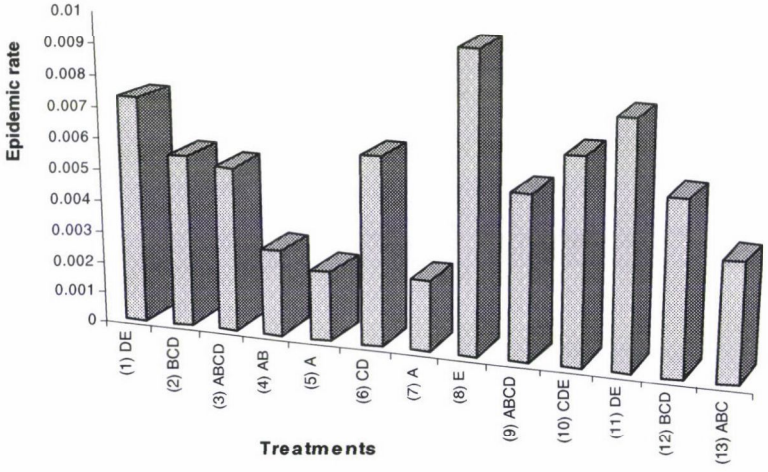


Fig. 3. Epidemic rate for plants treated with *A. solani* and the antagonists. 1. *Alternaria solani;* 2. *Penicillium* sp. a + *A. solani;* 3. *A. alternata* + *A. solani;* 4. *Fusarium semitectum* + *A. solani;* 5. *Chaetomium globosum* + *A. solani;* 6. *Aspergillus niger* + *A. solani;* 7. *Cryptococcus luteolus* + *A. solani;* 8. *Trichoderma harzianum* + *A. solani;* 9. *Penicillium* sp. b + *A. solani;* 10. *Cladosporium cladosporioides* + *A. solani;* 11. *T. polysporum* + *A. solani;* 12. *Nigrospora* sp. + *A. solani;* 13. *Epicoccum purpurascens* + *A. solani*. Columns followed by the same letter do not differ significantly according to Tukey's test ($P<0.05$)

The bibliographic review shows that the biocontrol of *Alternaria solani* has not been appropriately regarded and that research has been limited to only a few agents (Kumar and Singh, 1983; Okasha et al., 1989; Flood and Rees, 1986). The results of the current work clearly show that the antagonist capacity of some agents against *Alternaria* sp. is still unknown and that their high potential as biological control agents should be recognized.

Biological methods are real alternatives for the control of foliar diseases, but their success will depend on a knowledge of environmental conditions on the leaf surface as well as on an exhaustive understanding of pathogen and antagonist characteristics and the interactions between them.

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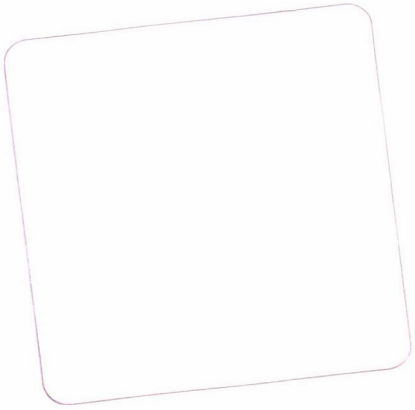

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EFFECT OF FERTILIZERS ON THE PRODUCTIVITY AND NPK REMOVAL OF A RICE-WHEAT CROPPING SYSTEM

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Field experiments were made on a sandy clay loam Fluvent soil to study the direct effects of fertilizers applied to rice and their residual effect on succeeding wheat in a rice-wheat cropping system during the crop years 1992–93 and 1993–94 at the Indian Agricultural Research Institute, New Delhi. Application of nitrogen up to 180 kg N ha^{-1} significantly increased most yield attributes and the grain and straw yield of rice. A significant residual effect on succeeding wheat was also recorded. P, K and Fe fertilization had neither a direct effect on rice nor a residual effect on succeeding wheat. Zinc application had a beneficial effect on some yield attributes and increased the rice grain and straw yield significantly in the second year of the study; there was no residual effect on the wheat yield. The application of N to rice significantly increased the NPK uptake by rice and also the N and P uptake by succeeding wheat. The rice-wheat cropping system resulted in the removal of 257 to 406 kg NPK ha^{-1} yr^{-1} (107 to 167 kg N, 15 to 26 kg P and 135 to 214 kg K), much more than the amount of these nutrients applied by the farmers. For producing 1 ton of grain 20 kg N, 3 kg P and 25 kg K were removed from the soil. A rice-wheat cropping system producing 5–8 t ha^{-1} yr^{-1} grain or even more thus highly depletes the soil of primary nutrients and carefully balanced fertilization is essential for sustained production.

Key words: rice-wheat, nutrient (NPK) removal, sustainability

Introduction

Rice-wheat is the most important two crops-a-year intensive cropping system in Asia (Fujisaka et al., 1994; Hobbs, 1994). About 12 million hectares of this system exist in four countries of South Asia (Woodhead et al., 1994) and about 9 million hectares in China (Huke et al., 1994). The productivity of this cropping system varies from 5 to 10 metric tonnes of grain ha^{-1} yr^{-1}. However, of late the soils and the system have shown signs of fatigue and there is a general decline in yields (Yadav et al., 1998) and in the efficiency of the fertilizer applied (Yadav, 1998). Micronutrient (Zn, Fe) deficiencies in the soil are considered as one factor responsible for this (Rattan et al., 1997; Takkar, 1996).

Dobermann et al. (1998) reported that for each metric ton of rice grain harvested, the crop removed 10–31 kg N, 8–35 kg P and 23–255 kg K ha^{-1}. Joseph and Prasad (1992) reported that the harvested wheat grain removed 28 kg N, 4.4 kg P, 41 kg K, 100 g Zn and 400 g Fe ha^{-1}. Data for the rice-wheat cropping system as a whole have not previously been reported. When both rice and wheat are heavily fertilized it leads to the nitrate pollution of the groundwater (Singh et al., 1995). In the present study, therefore, attempts were made to grow rice with a full level of fertilization while wheat was sown to utilize the residual fertility.

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Materials and methods

Field experiments were made at the Indian Agricultural Research Institute, New Delhi during the crop years (July–June) of 1992–93 and 1993–94 to study the direct effects of fertilizer applied to rice and its residual effects on succeeding wheat. The soil of the experimental field was a sandy clay loam Fluvent with pH 8.5 (1:2.5 soil to water), 11,000 kg ha^{-1} organic C (Walkley-Black), 21.6 kg ha^{-1} 0.5 M $NaHCO_3$-extractable P and 318 kg ha^{-1} 1 N NH_4OAc-exchangeable K at the start of the study, determined using the procedures of Prasad (1998). A rice-wheat cropping system had been practised on this field for the last 15 years.

The fertilizer treatments were 3 levels of N (60, 120, 80 kg ha^{-1}) and four combinations of NPK, Zn and Fe: 120 kg N + 26 kg P ha^{-1} (NP), NP + 48 kg K ha^{-1} (NPK), NPK + 25 kg $ZnSO_4$ ha^{-1} and NPK + 2 foliar sprays of 0.1% $FeSO_4.7H_2O$, together with a non-fertilized control. The 8 fertilizer treatments were randomized in blocks, with 3 replications. Nitrogen was applied as prilled urea in 3 applications, half at transplanting of rice, a quarter 20 days after transplanting (20 DAT) and the remainder at the panicle initiation stage (40 DAT). All P (as ordinary superphosphate), K (as muriate of potash) and Zn was broadcast on the plots and incorporated into the soil just before transplanting. Foliar sprays of iron were given at 20 and 40 DAT. Wheat was grown to study the residual effects, so no fertilizer was applied. However, since wheat growth was poor in 1993–94 all plots received 40 kg N ha^{-1} at the first irrigation.

The plots (5 m × 2.45 m) were separated on all sides by 0.5 m channels with 25 cm wide and 20 cm high bunds. Two or three seedlings of the rice variety Pusa Basmati-1 were transplanted per hill. The rice was transplanted on 13 July in 1992 and on 28 June in 1993. The crop was irrigated frequently to allow 3–5 cm standing water except when fertilizer N was applied. Water was finally withheld 15 days before harvesting.

After harvesting (November) the rice land was irrigated, allowed to come to condition, disced twice and levelled. Wheat (HD2285; a semi-dwarf variety) was sown on 11.12.92 and 26.12.93 and irrigated at the crown root initiation, active tillering, boot, flowering, grain filling and dough stages.

Five plants in each plot were selected at random for studies on yield attributes. Productive tillers were counted at ear emergence. At harvest samples of grain and straw were collected for plant nutrient analysis.

Wheat grain and straw samples were dried, finely ground and analysed for N, P, K using the procedures described by Prasad (1998). Zn was determined using an Atomic Absorption Spectrometer. Nutrient uptake was computed using the data on grain and straw yield and their nutrient concentrations.

Results

Direct effects on rice

Each increment of 60 kg N ha^{-1} significantly increased the productive tillers $hill^{-1}$ up to the highest level of N studied in both the years (Table 1). There was no significant effect of P, K or Fe fertilization on the number of productive tillers, but the application of Zn significantly increased it.

In 1992–93 each increment of 60 kg N ha^{-1} increased the number of grains $panicle^{-1}$ up to 180 kg N ha^{-1}. A significant effect of fertilizer treatments on 1000 grain weight was noted only in 1992–93, when 60 kg N ha^{-1} significantly increased it over the control and 120 kg N ha^{-1} over 60 kg N ha^{-1}. The effect of P, K, Zn and Fe on 1000 grain weight was not significant.

Table 1
Effect of fertilizer treatment on productive tillers, grains panicle^{-1} and yields in rice

Treatment	Productive tillers (No. hill^{-1})		Grains panicle^{-1}		Grain t ha^{-1}		Straw t ha^{-1}	
	1992–93	1993–94	1992–93	1993–94	1992–93	1993–94	1992–93	1993–94
Control	4.2	5.9	64.9	52.5	2.4	2.4	3.7	4.8
N_{60}	5.0	8.3	79.1	68.2	3.0	3.0	4.5	6.4
N_{120}	6.3	8.4	91.9	89.3	3.5	3.8	5.3	7.0
N_{180}	7.4	8.1	97.5	98.1	4.0	4.5	6.2	10.0
NP*	6.1	7.4	93.5	97.3	3.5	4.1	5.3	8.4
NPK*	6.3	8.7	93.5	101.3	3.6	4.3	5.5	9.3
NPK Zn*	6.2	8.8	98.2	116.4	3.8	4.9	5.6	10.7
NPK Fe*	6.6	7.3	93.7	99.1	3.6	4.3	5.5	9.3
SEm±	0.16	0.61	1.41	2.43	0.08	0.12	0.12	0.28

*N = 120 kg N ha^{-1}; P = 26 kg P ha^{-1}; K = 48 kg K ha^{-1}; Zn = $ZnSO_4$ 25; kg ha^{-1}
Fe = 0.1% $FeSO_4.7H_2O$ foliar spray, twice

Table 2
N, P, K and Zn uptake (kg ha^{-1}) by rice (grain + straw) as influenced by fertilizer treatments

Fertilizer treatment	Nitrogen		Phosphorus		Potassium		Zinc	
	1992–93	1993–94	1992–93	1993–94	1992–93	1993– 94	1992–93	1993–94
Control	46.6	49.7	7.1	6.5	59.6	77.4	269.6	292.9
N_{60}	60.4	68.3	9.9	10.3	78.1	106.4	348.0	405.4
N_{120}	71.6	78.8	12.6	13.9	92.6	116.2	430.0	487.7
N_{180}	83.8	102.6	15.2	18.3	102.5	164.7	502.7	661.2
NP	72.7	90.8	13.3	17.0	91.4	140.8	431.5	559.7
NPK	75.7	95.6	14.6	18.8	95.9	149.5	459.8	632.2
NPK Zn	78.5	116.8	12.7	16.8	101.7	172.1	528.4	813.7
NPK Fe	77.0	97.6	13.6	17.8	95.9	149.1	460.8	623.7
SEm±	2.01	2.98	0.39	0.53	2.51	5.80	11.7	19.0

Treatment details: See Table 1

A significant increase in the grain and straw yield of rice was recorded for each increment of 60 kg N ha^{-1} up to the highest level studied (Table 1). A significant increase in the grain yield of rice due to the combined application of P and K over N alone was recorded in the second year of the study (1993–94), when the application of P as well as K also increased the straw yield significantly. Zn application showed a significant increase in the grain and straw yield only in the second year of the study.

In both years nitrogen application significantly increased N uptake in both the grain and the straw up to 180 kg N ha^{-1} (Table 2). The total N uptake by the crop was therefore also significantly increased due to N application up to 180 kg N ha^{-1}. The application of P, K and Zn also increased N uptake by rice due to their effects on the grain yield.

The effect of fertilizer treatments on P uptake was more distinct in the grain than in the straw, since P migrates to the grain (Table 2). Thus N application, which increased the grain yield of rice, also resulted in a significant increase in P uptake in the grain in both years. A significant increase in P uptake by the straw was also noted when higher rates of N were applied. The application of P resulted in a significant increase in P uptake by both rice grain and straw. On the other hand, the application of Zn resulted in a significant reduction in P uptake in the grain and this was also perceptible in the total P uptake by the crop. There was no significant effect of K and Fe application on P uptake.

In contrast to the P uptake, the effects of the K fertilizer treatments were more distinct in the straw, since K migrates to the leaves as the grain forms (Table 2). Nitrogen applications significantly increased the K uptake in both grain and straw and therefore the total K uptake by the crop. K application, which was only 48 kg ha^{-1} yr^{-1}, did not significantly increase the K uptake by rice. The highest K uptake was recorded when the crop received 180 kg N ha^{-1} or NPK Zn application; these were the treatments that gave the highest grain and straw yields.

Nitrogen application significantly increased the Zn uptake by the grain and straw in both years. Zn application significantly increased the Zn uptake by rice in both grain and straw and the highest Zn uptake was recorded in the NPK Zn plot.

Residual effects on succeeding wheat

The application of N to rice left a significant residual effect on succeeding wheat, seen in the ears m^{-1} and grains ear^{-1}, but not in 1000-grain weight (Table 3). The number of ears m^{-1} was significantly greater when N was applied to rice, especially at higher rates of 120 and 180 kg N ha^{-1}. In 1992–93, the application of P to rice also resulted in significantly more ears m^{-1} in succeeding wheat. Ear length and grain ear^{-1} in wheat were significantly greater after N application to rice; the residual effects were more distinct at higher rates of N application.

A significant residual effect of fertilizer application to rice on wheat was observed only in the grain yield in 1993–94, when N application to rice resulted in a significant increase (Table 3).

Nitrogen application to rice resulted in a significant increase in the total N and P uptake by succeeding wheat (Table 4) in both the years. There was no significant residual effect of fertilizer applications to rice on the K S uptake by succeeding wheat.

Discussion

Of the five plant nutrients (N, P, K, Zn and Fe) studied, the effects of nitrogen were most marked, followed by that of Zn in the rice-wheat cropping system. In rice, nitrogen application increased productive tillers m^{-2}, panicle length, grains $panicle^{-1}$ and grain and straw yield. These results are thus in

Table 3
Yield attributes and yields of wheat as affected by fertilizer treatments in preceding rice

Fertilizer treatment	Ears m^{-1}		Grains ear^{-1}		Grain yield (t ha^{-1})		Straw yield (t ha^{-1})	
	1992–93	1993–94	1992–93	1993–94	1992–93	1993–94	1992–93	1993–94
Control	64.9	78.2	32.7	34.4	2.2	3.3	2.3	5.3
N_{60}	73.2	83.1	36.1	38.6	2.5	3.6	3.5	5.5
N_{120}	78.3	84.4	37.0	39.5	2.5	3.5	2.9	5.3
N_{180}	91.0	90.2	37.0	40.2	2.6	3.6	3.1	5.3
NP	79.9	86.2	37.2	39.6	2.2	3.7	3.4	5.8
NPK	83.3	88.0	36.7	40.0	2.6	3.7	3.0	5.8
PK Zn	85.9	89.4	37.8	39.7	2.3	3.7	3.1	5.6
NPK Fe	81.1	89.3	36.8	39.5	2.3	3.7	2.6	5.5
SEm±	2.16	2.86	0.86	1.10	0.19	0.13	0.29	0.23

Treatment details: see Table 1.

Table 4
NPK uptake (kg ha^{-1}) by wheat as affected by fertilizer treatments in preceding rice

Fertilizer treatment	Nitrogen		Phosphorus		Potassium	
	1992–93	1993–94	1992–93	1993–94	1992–93	1993–94
Control	44.7	75.5	6.2	10.9	41.3	91.8
N_{60}	53.3	84.2	7.2	12.0	59.0	95.8
N_{120}	52.1	82.4	7.2	11.6	50.8	92.9
N_{180}	54.8	83.5	7.3	11.4	53.3	92.0
NP	50.5	89.0	6.8	12.6	57.5	97.5
NPK	55.7	87.4	8.0	12.8	53.5	97.1
NPK Zn	50.8	88.7	6.9	12.5	53.6	101.1
NPK Fe	48.1	86.3	6.8	12.9	46.5	96.8
SEm±	3.02	3.19	0.48	0.45	4.21	4.03

Treatment details: see Table 1

accord with earlier studies on high-yielding varieties of rice (Lakhdive and Prasad, 1970; Rajale and Prasad, 1973). In the present study the highest yield of rice was obtained with the highest level of N studied, i.e. 180 kg N ha^{-1}; this was higher than that obtained with the combined application of 120 kg N, 26 kg P and 48 kg K ha^{-1}. This explains why farmers in the rice-wheat belt of India lay most emphasis on N application. Phosphorus application is restricted to wheat and K is generally not applied (Tandon, 1995). Another important finding of this study is the response of rice up to 180 kg N ha^{-1}; most earlier studies reported a response up to 100 or 120 kg N ha^{-1}, which is the general fertilizer recommendation at present (Tandon, 1995). Since the present study was made on a field where rice-wheat cultivation had been practised for 15 years, the response to and need for a higher level of N application with time is an indication of the decreased efficiency of N, as reported by Yadav (1998). Most farmers in the rice-wheat belt are gradually increasing their rate of N application despite warnings of nitrate pollution of shallow well waters in Punjab (Singh et al., 1995).

The benefits of Zn fertilization were recorded in the yield attributes as well as in the grain and straw yield of rice. Most farmers apply 10–25 kg $ZnSO_4$ ha^{-1} which is also the current recommendation (Rattan et al., 1997).

Due to its effect on grain and straw yield, N application also resulted in increased N and PK uptake by rice. Phosphate application resulted in increased P uptake by rice. Zn application also increased the N and K uptake by rice but decreased the P uptake, indicating a negative Zn and P interaction, as reported by Takkar et al. (1976), Tiwari and Pathak (1978) and Prasad and Power (1997).

Only the N application to rice had a significant residual effect and resulted in higher yield attributes and grain and straw yields in wheat. This should be taken into account when recommending N application in wheat succeeding rice for economising on fertilizer N use, as is practised for cropping with winter wheat in the U.K. (Bhogal et al., 1997). At present this is ignored and both rice and wheat are heavily fertilized with N (Tandon, 1995).

From the data on grain yield and NPK uptake by rice and wheat, the productivity and NPK uptake yr^{-1} of rice-wheat cropping systems was computed (Table 5). The productivity of rice-wheat cropping systems varied from 5.8 t ha^{-1} yr^{-1} in the control to 8.2 t ha^{-1} yr^{-1} in plots receiving 180 kg N ha^{-1} in rice. These are values generally obtained by farmers in the rice-wheat belt. Similar values were reported by Singh and Verma (1998) and Hegde (1998).

Table 5

Productivity (t ha^{-1} yr^{-1}), NPK removal (kg ha^{-1} yr^{-1} and kg t^{-1} grain) by a rice-wheat cropping system

Fertilizer treatment	Grain* (t ha^{-1} yr^{-1})	Nitrogen (kg ha^{-1} yr^{-1})	Phosphorus (kg ha^{-1} yr^{-1})	Potassium (kg ha^{-1} yr^{-1})	N+P+K (kg ha^{-1} yr^{-1})
Control	5.8	107.3	15.3	135.0	257.6
		(18.5)**	(2.6)	(23.3)	(44.4)
N_{60}	6.8	133.6	19.8	169.5	322.9
		(19.8)	(3.0)	(24.9)	(47.5)
N_{120}	7.4	141.9	22.7	176.2	340.8
		(19.2)	(3.1)	(23.8)	(46.1)
N_{180}	8.2	162.3	26.1	206.3	394.8
		(19.8)	(3.2)	(25.1)	(48.1)
NP	7.3	151.5	24.9	193.6	370.0
		(20.8)	(3.4)	(26.3)	(50.5)
NPK	7.7	157.3	26.7	198.0	382.0
		(20.5)	(3.5)	(25.6)	(49.6)
NPK Zn	7.7	167.4	24.5	214.1	406.0
		(21.7)	(3.2)	(27.8)	(52.7)
NPK Fe	7.6	154.6	25.6	194.6	374.8
		(20.5)	(3.4)	(25.7)	(49.6)

• Rice+Wheat; **Values in parenthesis are kg nutrient removed per metric ton (t) grain

The removal of primary nutrients by the rice-wheat cropping system was 258 to 406 kg NPK (107–167 kg N, 15–26 kg P and 135–206 kg K ha^{-1} yr^{-1}) for a production range of 5.8 to 8.2 t ha^{-1} yr^{-1}. This nutrient removal is nearly 1.5 times the amount of fertilizer applied to the rice-wheat cropping system by the farmers (120 kg N + 13 kg P and 24 kg K ha^{-1} for each rice and wheat crop) (Tandon, 1995). The removal of NPK per metric ton of grain was about 20 kg N, 3 kg P and 25 kg K ha^{-1} totalling 48 kg of N+P+K. These results are in agreement with those reported by Dobermann et al. (1998) for rice and Joseph and Prasad (1992) for wheat. Thus, the present results show that the rice-wheat cropping system is highly productive, but also highly nutrient depleting, and for sustained production adequate fertilization is essential. A consideration of the residual effect of the nitrogen applied to rice could reduce the nitrogen load in this cropping system.

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PHOSPHATE SORPTION–DESORPTION OF CHARACTERISTIC GREEK SOILS

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In this work the sorption and desorption of phosphate was studied in four types of Greek soils at five different P concentrations and three temperatures. The phosphate sorption data at 5°, 25° and 50°C were fitted to the Temkin, Freundlich and Langmuir adsorption isotherms and the equilibrium sorption parameters were determined. They were found to be closely correlated to organic matter, clay content, total P, oxalate extractable Al, calcium carbonate, C.E.C and dithionite-extractable Fe at 5° and 25°C; to pH, active calcium, oxalate extractable Fe and Mn at 5°C and to Olsen P and dithionite-soluble Fe at 50°C. The P sorption capacity in 6.45 μmol P l^{-1} solutions of the soils varied widely in the order Alfisol > Vertisol > Entisol > Inceptisol at 5°C, Alfisol > Vertisol > Inceptisol > Entisol at 25°C and Alfisol >Entisol >Inceptisol >Vertisol at 50°C. Desorption data at 5° and 25°C indicated the low desorbability of sorbed P. The kinetic P sorption data at 25°C were fitted to a modified Freundlich type kinetic equation to obtain a parameter related to the sorption rate coefficient.

Keywords: phosphate, sorption, desorption, Greek soils

Introduction

Entisol, Alfisol, Inceptisol and Vertisol are the four major types of Greek soils suitable for olive trees, grain, vineyards and cotton plantations. According to Lin et al. (1983) adsorption prevails at low phosphate concentration in the soil solution and precipitation at higher phosphate concentrations. Mehadi and Taylor (1988) considered that adsorption and precipitation reactions were involved over a short period during phosphate fixation in the soil. Dimirkou (1993), Dimirkou et al. (1996) and Ioannou et al. (1996a, b, c) considered the water:soil ratio, the temperature, the $CaCO_3$ content, clay minerals such as kaolinite and bentonite, and iron oxide to be factors governing the P concentrations in soil solutions. In soils fertilized recently with phosphates unstable reaction products are changed into more stable ones through further crystallization (Sanyal and De Datta, 1991). In these soils the chemical mobility of the phosphates is not controlled by phosphates of Al and Fe, such as variscite and strengite (Ryden and Pratt, 1980).

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The purpose of the present study was to improve phosphate management strategies in the four major types of Greek soils (Alfisol, Entisol, Inceptisol and Vertisol) by studying the sorption and desorption at three different temperatures and the kinetics of phosphates at 25°C in Alfisol, Entisol, Inceptisol and Vertisol soils.

Materials and methods

Soil samples (0–30 cm) were collected from olive, grain, vineyard and cotton fields in Central Greece. The classification of the soils was done according to the Soil Taxonomy of USDA (1975) (Table 1). Samples were collected, dried and crushed to pass through a 2 mm sieve to form the initial soil samples.

Table 1
Some characteristics of the soils studied

Soils*	1	2	3	4	5	6	7	8	9	10	11	12	13
Entisol[a]	7.46	1.2	55	49.6	10.0	15.18	328	4.5	111	178	67	0.063	19.2
Inceptisol[b]	7.56	1.4	63	33.6	16.6	18.80	371	7.0	3.2	314	118	0.071	20.0
Vertisol[c]	7.50	1.6	66	4.2	2.2	17.33	371	6.0	447	891	704	0.241	30.8
Alfisol[d]	7.29	2.3	59	15.2	10.0	14.70	478	7.5	550	1031	486	0.336	27.6

*Taxonomic classification: [a]Xerorthent; [b]Xerochrept; [c]Chromoxerert; [d]Haploxeralf
1: pH in H_2O (1:2); 2: Organic matter (%); 3: Clay (%); 4: Total $CaCO_3$ (%); 5: Active Ca (%); 6: Exchangeable Ca + Mg (cmol kg^{-1}); 7: Total P (mg kg^{-1}); 8: Olsen P (mg kg^{-1}); 9: Ammonium oxalate Fe (mg kg^{-1}); 10: Ammonium oxalate Al (mg kg^{-1}); 11: Ammonium oxalate Mn (mg kg^{-1}); 12: S.D.C.P.H. Fe (%); 13: C.E.C. (cmol kg^{-1})

Soil pH was measured in a 1:2 (w/v) soil/water mixture using a combination electrode. The organic matter in the soil was measured by the method of Walkley and Black (Nelson and Sommers, 1982). The clay content was determined by the Bouyoucos hydrometer method. Total $CaCO_3$ was measured with a Bernard calcimeter after HCl treatment. Exchangeable calcium was determined by the permanganate titration procedure after extraction with 1N NH_4OAc. Cation Exchange Capacity (C.E.C.) and exchangeable calcium plus magnesium were measured by the Papanicolau (1976) method. Total phosphorous was determined by the digestion method (Olsen and Dean, 1965), while assimilative P was determined in sodium bicarbonate by the method of Olsen et al. (1954).

Oxalate-extractable Fe, Al and Mn were determined by A.A.S. after extraction with ammonium oxalate-oxalic acid buffer, pH 3.0 (Schwertmann, 1964), and dithionite soluble-Fe was determined by A.A.S. after extraction with dithionite-citrate pentahydrate (Holmgren, 1967).

Phosphate sorption-desorption equilibrium experiment

Three g of soil were added to a 50 ml centrifugal tube that already contained 30 ml of 0.01 M $CaCl_2$ solution and different concentrations of phosphorus (277–3600 μg P/30 ml, as KH_2PO_4). The centrifugal tubes were put in a shaker and shaken twice a day for 30 minutes for 10 to 40 days (depending on the equilibrium time that each type of soil required). Tests were previously made for each soil type to calculate the equilibrium time of P (Fox and Kamprath, 1970). One drop of toluene was added to each tube to avoid the growth of microorganisms during the waiting time for the equilibrium. Centrifugation of the samples took place after the end of the equilibrium time and the quantity of P in the supernatant (28 ml) was calculated according to Murphy and Riley (1962).

The concentration of P adsorbed from each sample was found by subtracting the concentration of P in the supernatant from the concentration of P that was added at the beginning. The above experiment was repeated three times for each soil type at three different temperatures (5°, 25° and 50°C).

The desorption of P from each soil type in this study was determined from samples of adsorption without the supernatant after the centrifugation procedure. Twenty-eight ml of 0.01 M $CaCl_2$ solution were added to the samples and the Roy and De Datta (1985) method was used. The samples were shaken twice a day for 30 minutes for 10 to 40 days. After centrifugation the quantity of P in the supernatant (30 ml) was calculated according to Murphy and Riley (1962). The amount of P desorbed for each type of soil was calculated by subtracting the P concentration in the 2 ml that remained from the adsorption, from the quantity of P found in the supernatant.

Data obtained for sorbed P were fitted to the following adsorption isotherm equations:

Temkin equation (Hayward and Trapnell, 1964)

$$X = k_1 \ln (k_2 C) \tag{1}$$

where X is the amount of P sorbed by unit weight of soil, C is the equilibrium P concentration, k_1 and k_2 are fitted constants;

Freundlich equation:

$$X = KC^{1/n}, (n>1)$$

or

$$\log X = \log K + \frac{1}{n} \log C \tag{2}$$

where K and n are fitted constants, logK is the intercept and 1/n the slope of the line given by plotting logX as a function of logC;

Langmuir equation:

$$\frac{C}{X} = \frac{1}{K_1 K_2} + \frac{C}{K_1} \tag{3}$$

where K_1 and K_2 are constants, $1/K_1K_2$ is the intercept and $1/K_1$ is the slope of the line given by plotting C/X as a function of C. K_1 is designated as the Langmuir sorption maximum, and K_2 is related to binding energy.

Data obtained for desorbed P were fitted to Temkin $\{Xd=K_{1d}\ln(K_{2d}C)\}$ and Freundlich $\{Xd=K_dC^{1/n}, (n>1)\}$ equations, where Xd is the amount of P desorbed by unit weight of soil and K_{1d}, K_{2d} and K_d are fitted constants.

Phosphate sorption kinetic experiment

Three- gram samples of Vertisol and Inceptisol soils with 30 ml of 0.01 M $CaCl_2$ solution containing various amounts of KH_2PO_4 (277–3600 µg P/30ml) and two drops of toluene were added to 100 ml centrifugal tubes. These centrifugal tubes were put into a waterbath (with shaker and thermostat) and shaken at 25°C for 15 minutes to 21 hours. At the end of each predefined period (15, 20, 30, 45 min, 1, 2, 3, 4, 6, 9, 10, 12, 18, 19, 20, 21 h), the samples were centrifuged and the concentration of P was determined according to Murphy and Riley (1962).

The experimental data of this procedure were fitted to the modified Freundlich equation (concerning kinetics) according to Kuo and Lotse (1974).

$$X = Ka\ Co\ t^{1/m} \tag{4}$$

$$\log X = \left[\log Ka + \log Co\right] + \frac{1}{m} \log t \tag{5}$$

where Co is the initial solution concentration of P, t is the time, m is a constant, and Ka is a constant related to the rate at which the given P sorption system approaches equilibrium.

Results and discussion

The physical and chemical characteristics of each type of soil are given in Table 1. As Table 1 shows, there is a narrow range of pH, C.E.C, clay, total and assimilative P and a wide range of total $CaCO_3$, exchangeable Ca, ammonium oxalate-soluble Fe, Al and Mn and dithionite-extractable Fe in the studied soils.

Figure 1 shows the plot of the Freundlich equation (2) and the simple linear correlation coefficient (r = 0.99) between the values of logX and logC of each soil at each studied temperature.

Figure 2 shows the plot of the Langmuir equation (3) and the simple linear correlation coefficient (r) between the values of C/X and C (r : 0.94–0.99) of each soil at each studied temperature. The comparison of the r values (Figs 1 and 2) shows that the Freundlich equation fits better than the Langmuir in the case of experimental results of adsorbed P for soils in equilibrium. This agrees with the results published by Ratkowsky (1986). This is probably because, although the Freundlich equation was given as an empirical equation, it implies that the affinity of adsorption decreases exponentially as the adsorption surface saturation increases, which seems much more logical than the hypothesis of the Langmuir equation for a constant energy bond inherent in the Langmuir equation (Sposito, 1984). Alternatively, the Freundlich is a log-log plot and variability is lost by taking logs.

Table 2 shows that the Freundlich K values during sorption and desorption, the Langmuir sorption maximum (K_1) and the binding energy (K_2) of P sorption by Alfisols, Inceptisols and Entisols increase as temperature increases. The K_1 and K_2 values are different for each soil type and underline their different behaviour as regards the adsorption of P.

The comparison of the Freundlich K values during sorption and desorption (Table 2) shows that the K values for Entisols and Vertisols at 50°C are much higher than at 25°C and 5°C. The K_2 and Freundlich K values during sorption and desorption for Vertisols at 25°C are lower than at 5°C and 50°C. The K_1 values at 5°C and 25°C are very close for Inceptisols and Entisols and at 25°C and 50°C for Alfisols. The K_1 and K_2 values are different for each soil type and underline their different behaviour as regards the adsorption of P. All the soils were also characterized by high P-binding energy (K_2), which was 1.4, 5.1, 2.9 and 0.78 times higher at 25°C) than at 5°C and 2.9, 1.5, 3.3 and 0.9 times lower than at 50°C for Alfisols, Inceptisols, Entisols and Vertisols, respectively. These observations explain the values of phosphorus desorbed by these soils.

The P sorption by the present soils at 6.45 μmol P l^{-1} in equilibrium solution at 5°, 25° and 50°C, which was used as the standard for comparing the P requirement of the soils (Loganathan et al., 1987), was calculated from the Freundlich isotherm (2) and the values are recorded in Table 2. A comparison of these values shows that Alfisols had the highest P requirement.

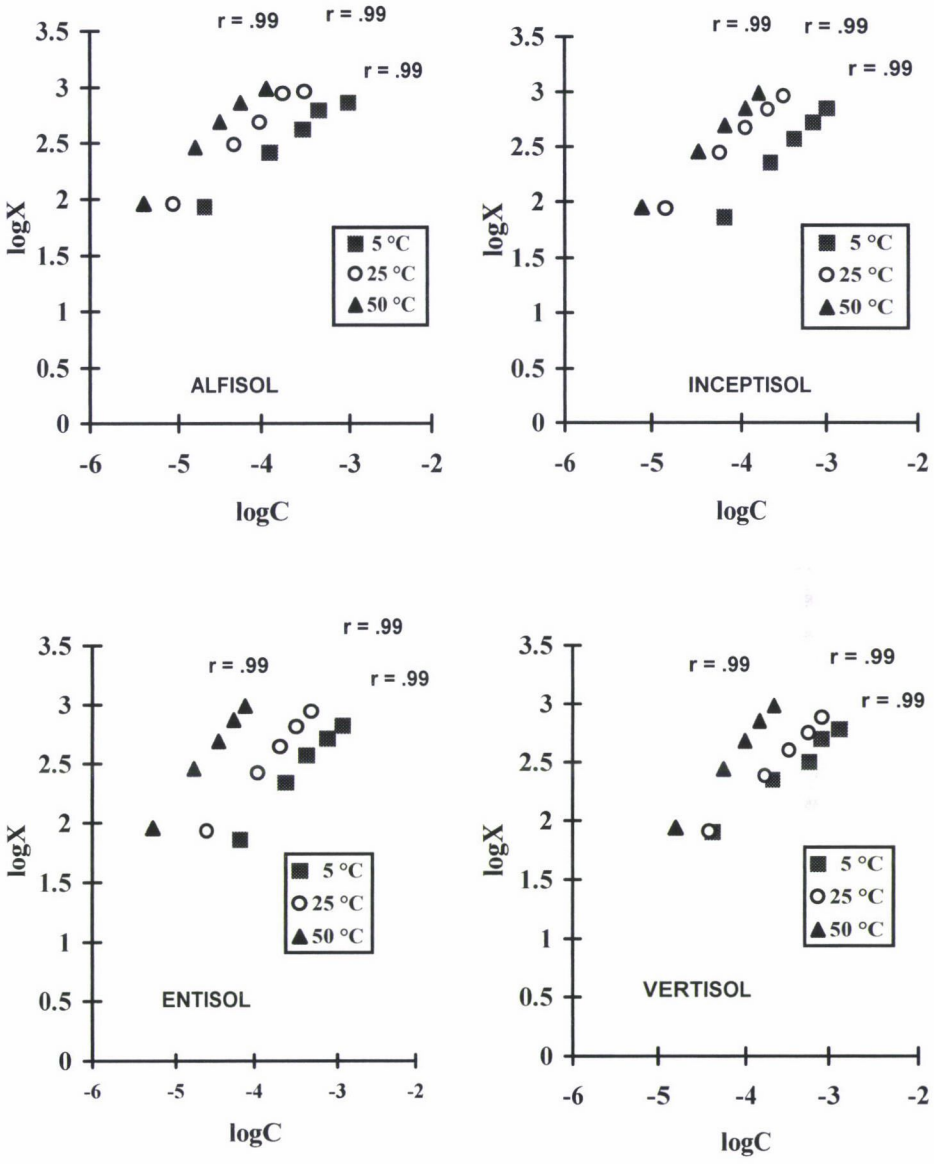

Fig. 1. Freundlich adsorption isotherms for P sorption by the four soils at different temperatures; X is the amount of P sorbed per unit weight of soil and C is the equilibrium P concentration (Equation 2)

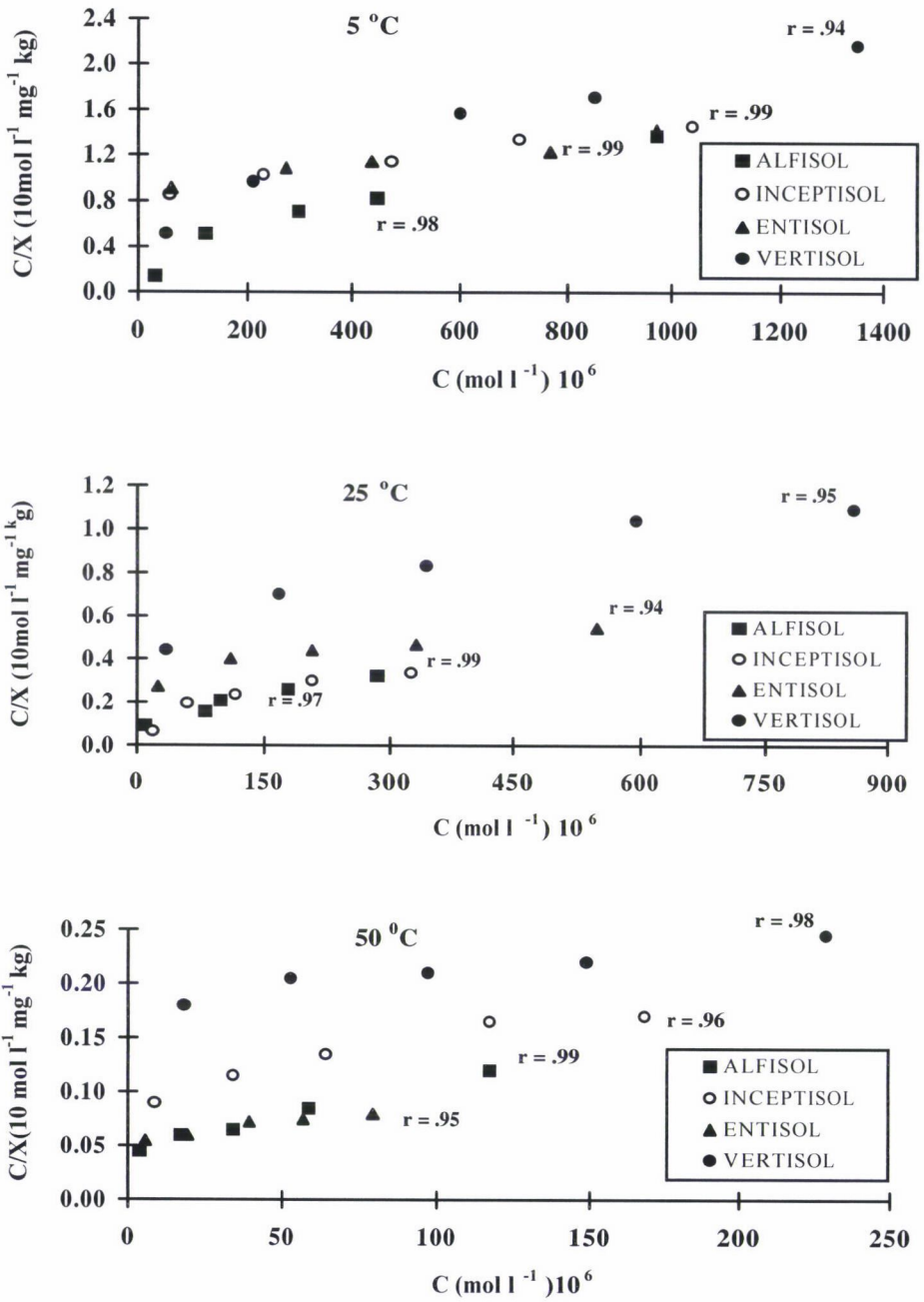

Fig. 2. Langmuir adsorption isotherms for P sorption by the four soils at different temperatures; X is the amount of P sorbed per unit weight of soil and C is the equilibrium P concentration (Equation 3)

Table 2
Equilibrium parameters at 5°, 25° and 50°C

Soil order	T°C	Freund. $K^{(1)}$ (mg P kg^{-1})	$K_1{}^{(2)}$ (mg P kg^{-1})	$K_2{}^{(3)}$ (l mol^{-1} P)	Freund. $K^{(4)}$ (mg P kg^{-1})	P sorption at 6.45 µmol/l in solution K (mg P kg^{-1})
Alfisol	5	46665.9	892.7	3702	1288	36.00
	25	269153.5	1583	5074	1359	75.16
	50	787045.8	1571	14720	20529	139.60
Inceptisol	5	191425.6	1627	714	1066	11.83
	25	429536.4	1659	3631	1777	50.10
	50	957194.0	1997	5406	11974	77.09
Entisol	5	143548.9	1967	555.3	2325	12.44
	25	351560.0	1920	1607	3088	28.70
	50	3863669.7	3289	5371	83462	113.76
Vertisol	5	26853.4	794	2053	780	27.66
	25	7177.9	1285	1464	713	61.09
	50	2218196.0	4153	1338	119×10^{12}	38.73

$^{(1)}$Freundlich K during adsorption, $^{(2)}$Langmuir sorption maximum
$^{(3)}$Binding energy, $^{(4)}$Freundlich K during desorption

Figures 3 and 4 give the Temkin plots for selected soils at 5°, 25° and 50°C during sorption and desorption. The experimental results from graph 3 give curves, not the straight lines expected from Equation 1. This observation is in accordance with that of Russell et al. (1988).

Table 3 shows the kinetic parameters for Vertisols and Inceptisols at 25°C. The K_a values were found to vary widely between the given soils. These values ranged from 162 to 295 mmol l^{-1} for Vertisols and from 184 to 258 mmol l^{-1} for Inceptisols. In Vertisols the K_a values decreased as the initial concentration values increased from 0.0 to 1.62 mmol l^{-1} and from 2.419 to 3.290 mmol l^{-1}. For Inceptisols the K_a values decreased as the initial concentration values increased from 0.298 to 0.952 mmol l^{-1} and from 1.629 mmol l^{-1} to 3.290 mmol l^{-1}. The above observations agree with those of Kuo and Lotse (1974) and Kato and Owa (1989) and can be explained by the Bronsted-Bjerrum activity rate theory for ionic reactions in dilute solutions (Sparks, 1986).

Soil properties related to total adsorption capacity

Significant positive or negative correlations were found between the Langmuir sorption maximum and total $CaCO_3$, dithionite-soluble Fe and exchangeable Ca^{2+} at 5°C , and total $CaCO_3$ and C.E.C. at 25°C (Table 4).

As Table 4 shows, total $CaCO_3$ at 5°C and 25°C, exchangeable Ca at 5°C, oxalate-extractable Al at 5° and 50°C and dithionite-soluble Fe at 5°C appear to play an important role in explaining the variability of P sorption in the studied soils.

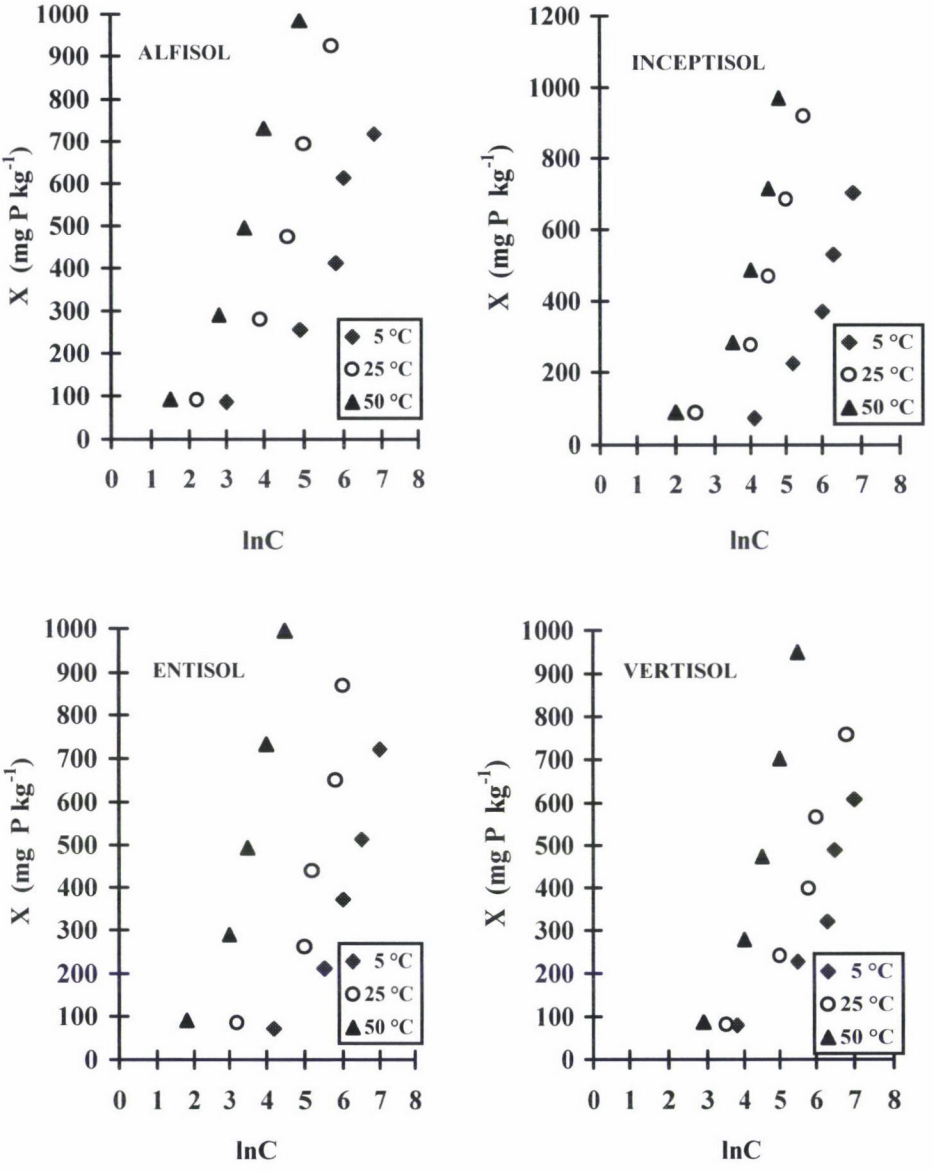

Fig. 3. Temkin adsorption isotherms for P sorption by the four soils at different temperatures; X is the amount of P sorbed per unit weight of soil and C is the equilibrium P concentration (Equation 1)

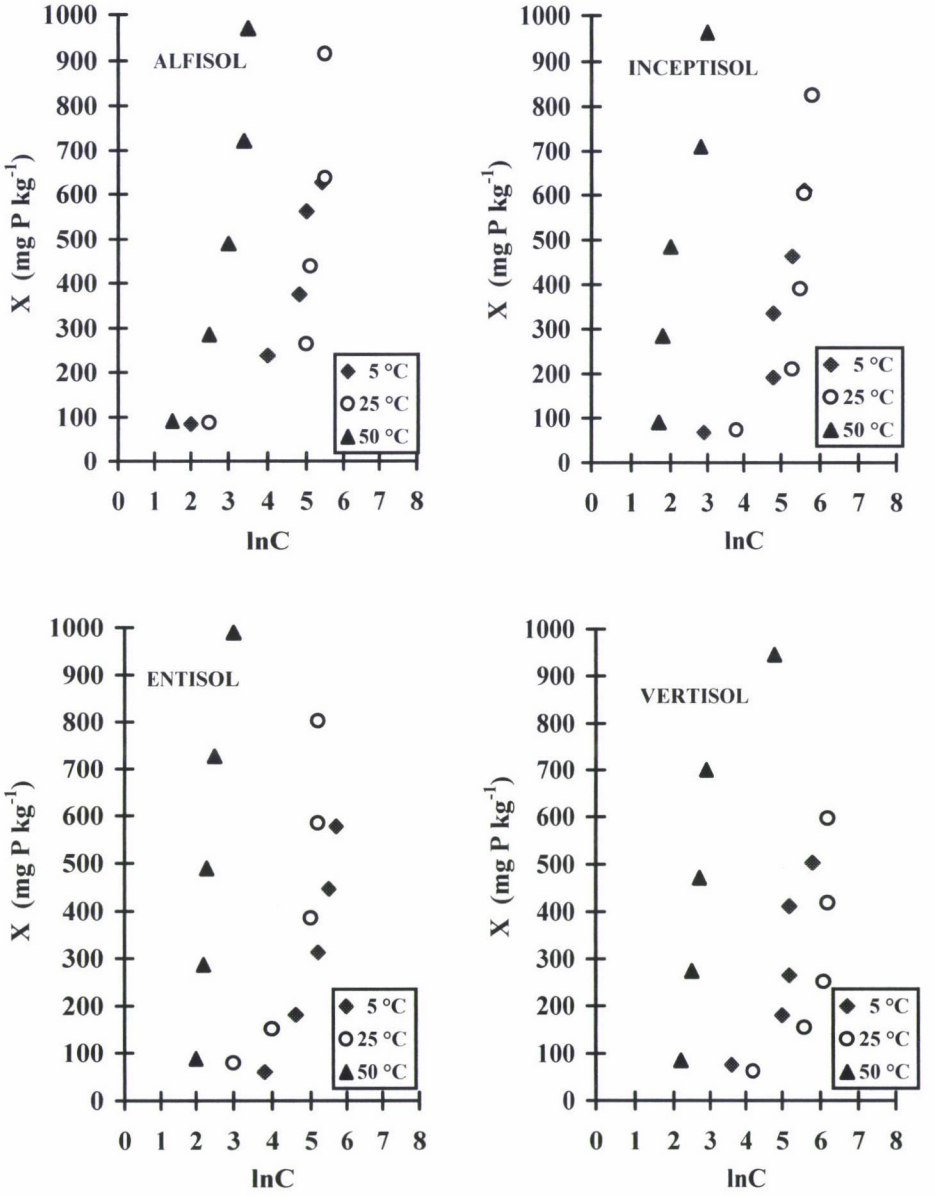

Fig. 4. Temkin adsorption isotherms for P sorption by the four soils at different temperatures during desorption; X is the amount of P sorbed per unit weight of soil and C is the equilibrium P concentration

Table 3
Kinetic parameters at 25°C for Vertisols and Inceptisols

Soil order	C_0 mmol.l^{-1}	log K_a	K_a mmol.l^{1}	m	r
Vertisol	0.298	2.275	188.36	14.08	0.914
	0.952	2.261	182.38	10.64	0.990
	1.629	2.210	162.18	10.00	0.990
	2.419	2.470	295.00	105.48	0.960
	3.290	2.460	288.00	101.20	0.977
Inceptisol	0.298	2.402	252.20	42.96	0.808
	0.952	2.347	222.50	37.04	0.832
	1.629	2.413	258.90	146.84	0.851
	2.419	2.331	214.50	72.99	0.716
	3.290	2.266	184.40	25.77	0.817

Table 5 shows that the Langmuir sorption maximum of P (K_1) per unit of organic matter decreased with increasing organic matter content at 5°C and 25°C but not at 50°C.

Soil properties correlated to the Freundlich sorption constant, K

Significant positive or negative correlations were observed between the Freundlich sorption, K (Table 4) and exchangeable Ca, C.E.C. and oxalate-extractable Mn at 5°C and 25°C, oxalate-extractable Fe and Al and dithionite-soluble Fe at 5°C, and assimilative P at 50°C.

Soil properties correlated to binding energy

Significant correlations were observed between binding energy (Table 4) and organic matter, oxalate-extractable Fe and Al and dithionite-soluble Fe at 5°C and total P at 5°, 25° and 50°C. A significant negative correlation was found with pH at 5°C.

The variability in the binding energy constant (Table 4) was more closely correlated to the relevant soil properties than the adsorption maxima of the soils. These results suggest that a portion of the binding energy is attributed to sites associated with oxalate-extractable Al and dithionite-extractable Fe. These observations are in agreement with those of Holford and Mattingly (1975). Although this equilibrium constant is considered as an equation coefficient with very little physical significance (Harter, 1984), it appears to be mainly associated with pH, organic matter, total P at 5°, 25° and 50°C and surfaces of Fe compounds or perhaps, more generally, with the amounts of all the hydrous oxides present in these soils at 5°C.

Soil properties correlated to Freundlich desorption, K

Significant correlations were observed between Freundlich desorption, K (Table 4) and total $CaCO_3$ at 5° and 25°C and dithionate-extractable Fe at 50°C. Significant negative correlations were found with clay at 5°C and 25°C.

The Freundlich desorption constant was correlated with clay at 5° and 25°C with total $CaCO_3$, C.E.C. and oxalate-extractable Al and Mn at 25°C, and with dithionite-extractable Fe only at 50°C.

Table 4
Simple linear correlation coefficient between P sorption-desorption parameters and relevant soil properties at 5°, 25° and 50°C

Sorption-desorption parameters	T°C	pH	Organic matter (%)	Clay	$CaCO_3$	C.E.C.	Total P	$P^{\times}$	Ca^{+2}♦	Fe*	Al*	Mn*	Fe%
Freundlich sorption K	5	0.563	-0.651	–0.298	0.826	–0.945	–0.513	–0.180	0.850	–0.962	–0.898	–0.932	–0.885
	25	0.085	–0.245	–0.565	0.803	–0.895	–0.092	0.057	0.955	–0.7087	–0.67	–0.916	–0.586
	50	0.221	–0.676	–0.479	0.518	–0.312	–0.795	–0.994	–0.344	–0.284	–0.528	–0.288	–0.492
Langmuir sorption maximum	5	0.56	–0.76	–0.04	0.98	–0.79	–0.31	0.14	0.91	0.78	0.83	0.74	–0.910
	25	0.30	–0.40	–0.72	0.96	–0.94	–0.63	–0.62	0.55	0.71	0.70	0.34	–0.624
	50	0.44	–0.52	0.27	–0.15	0.28	–0.66	–0.71	–0.76	–0.67	–0.86	–0.82	–0.170
Binding energy	5	–0.94	0.98	–0.18	–0.44	0.48	0.95	0.62	–0.08	0.93	0.94	0.89	0.983
	25	–0.70	0.76	–0.13	–0.22	0.13	0.91	0.83	0.42	0.26	0.39	0.26	0.466
	50	–0.88	0.79	–0.44	–0.07	0.08	0.86	0.58	0.30	0.47	0.40	0.26	0.537
Freundlich desorption K	5	–0.18	–0.39	–0.95	0.84	–0.67	–0.38	–0.70	0.22	–0.37	–0.62	–0.68	–0.47
	25	–0.08	–0.56	–0.90	0.97	–0.87	–0.51	–0.61	0.47	–0.65	–0.82	–0.88	–0.71
	50	0.27	0.51	0.73	–0.71	0.75	–0.50	–0.13	–0.85	–0.43	0.30	0.79	0.99
P sorption at 6.45 µmol/l	5	–0.768	0.918	0.219	–0.809	0.864	0.838	0.535	–0.531	0.981	0.977	0.828	0.999
	25	–0.545	0.914	0.486	–0.857	0.780	0.895	0.849	–0.235	0.795	0.929	0.752	0.905
	50	–0.766	0.437	0.826	–0.396	–0.314	0.491	0.135	–0.409	0.128	0.002	–0.383	0.185

×, assimilative P, ♦exchangeable Ca^{2+}, *ammonium oxalate-extractable

Table 5
Correlation between organic C content and Langmuir sorption maximum for P (K_1) per unit C content of the soils

Soil sample	Temperature °C	Organic C $g.kg^{-1}$	K_1/organic C
Entisol	5	6.96	282.60
Inceptisol	5	8.12	200.00
Vertisol	5	9.28	85.50
Alfisol	5	13.34	66.91
r			–0.83
Entisol	25	6.96	276.00
Inceptisol	25	8.12	204.30
Vertisol	25	9.28	137.90
Alfisol	25	13.34	118.60
r			–0.84
Entisol	50	6.96	472.50
Inceptisol	50	8.12	245.90
Vertisol	50	9.28	447.50
Alfisol	50	13.34	114.50
r			–0.92

Soil properties correlated to 6.45 μmol P l^{-1} in equilibrium solution

Significant correlations were observed between P sorption at 6.45 μmol P l^{-1} of solution at 5° and 25°C and organic matter, total P, oxalate-extractable Al and dithionite-extractable Fe and at 5°C with C.E.C. and oxalate-extractable Fe and Mn (Table 4). Significant negative correlations were observed with $CaCO_3$ at 5° and 25°C .

The above correlations suggest that organic matter, total P, $CaCO_3$, oxalate- extractable Fe and Al, and dithionite-extractable Fe are the soil properties affecting P sorption at 6.45 μmol P l^{-1} in solution at 5° and 25°C.

The significant correlation of organic matter with P sorption at 6.45 μmol P l^{-1} may be attributed to a possible blockage of sites associated with P sorption by organic anions (Fox and Kamprath, 1970).

The high correlation between P sorption at 6.45 μmol P l^{-1} and oxalate and dithionite-extractable Fe may be indicative of the reactivity of P and Fe compounds in these calcareous soils. The high correlation with extractable Al is in agreement with earlier observations on the role of Al in acid soils in fixing P from soluble fertilizers (Schalacha et al., 1972), and this study shows that even in these calcareous soils the role of Al in fixing P from soluble fertilizers at 5° and 25°C is very important.

The slight negative effect of pH on P sorption at 6.45 μmol P l^{-1} is also consistent with the negative effect of pH on P adsorption by goethite, goethite-bentonite, hematite, hematite-kaolinite and hematite-bentonite measured by Hingston et al. (1968), Dimirkou et al. (1996; 1998) and Ioannou et al. (1996; 1998). The correlation between soluble P and P sorption at 25°C proves that soluble P provides a good index of the P available in the soil solution for these calcareous soils (Fig. 5.).

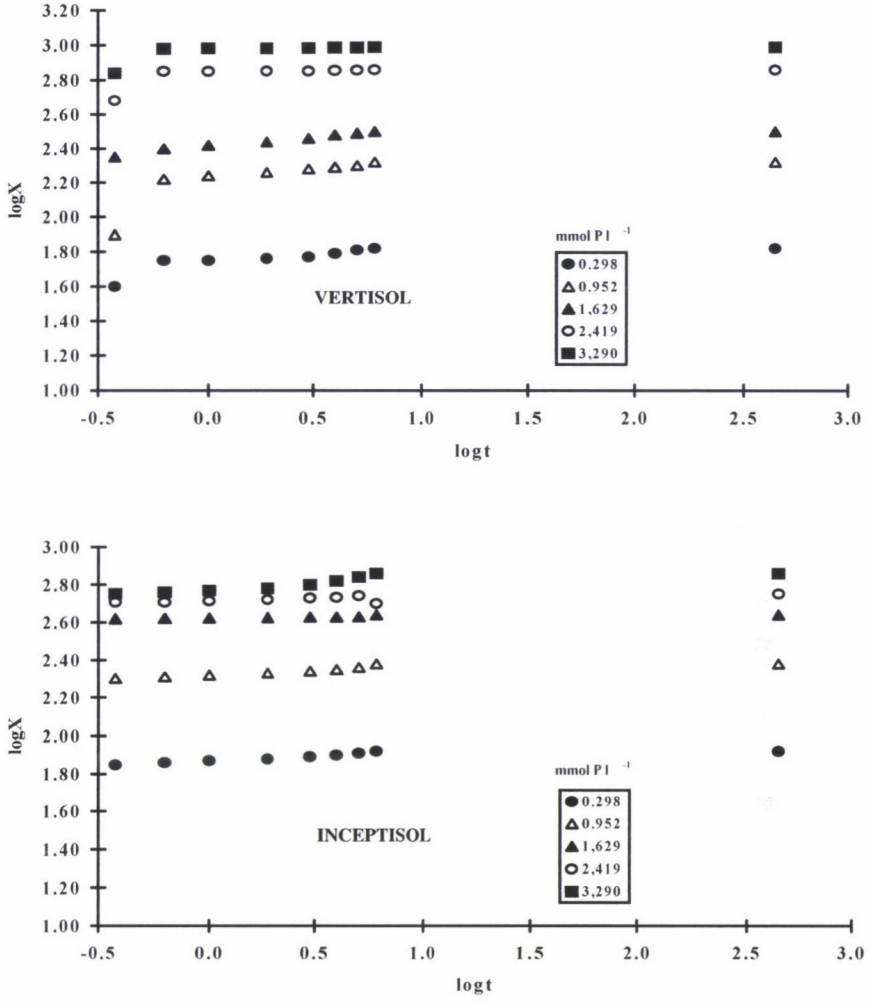

Fig. 5. Phosphate sorption (X) by Inceptisols and Vertisols at 25°C as a function of time (t) and initial phosphate concentration

Conclusions

The correlation established between P adsorption at 6.45 μmol P l^{-1} and oxalate-extractable Fe at 5° and oxalate-extractable Al and dithionite-extractable Fe at 5° and 25°C suggested that Fe and Al compounds provide the most surface responsible for high-energy adsorption and that hydrous oxides provide high-energy P sorption sites in these soils. These results fully support earlier evidence (Cole and Olsen, 1959) that hydrous oxides adsorb P even in calcareous soils. The negative correlation between $CaCO_3$ and P sorption at 6.45 μmol P l^{-1} at 5°

and 25°C is in agreement with previous reports of P buffering by soils (Webber and Mattingly, 1970). This can be explained by the smaller total surface area present in materials containing large quantities of carbonate.

The most important variables for accounting for the variability of the Langmuir sorption maximum and for P sorption at 6.45 μmol P l^{-1} in solution turned out to be organic matter, total $CaCO_3$, oxalate-extractable Al and dithionite-extractable Fe at 5° and 25°C, exchangeable, Ca^{2+} and oxalate extractable Fe at 5° and cation exchange capacity (C.E.C) at 25°C.

All the information given by this study may be incorporated into crop and environmental P management practices when planning an appropriate P source, rate, temperature and timing of application for a calcareous soil with known P sorption-desorption behaviour. Concern that the P saturation of sludge-treated agricultural land may result in possible leaching losses of P has resulted in legislation to control applications to soil in certain European countries (Smith, 1996). Thus, for soils with high sorption capacity and strong P-binding energy or low desorption rate, a less soluble P source should be chosen that releases P into the soil solution in smaller concentrations over a long period of time. This is expected to slow down the P fixation reaction in the given soils, and maintain fertiliser P in plant-available form for a long period. The sludge treatment of this agricultural land should be avoided or carried out very carefully since P saturation and leaching losses of P are very probable.

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EVALUATION OF TYPE CLASSIFICATION IN THE LIMOUSINE BREED

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Type classification based on four principal quality groups (utility score, score for length, score for width, score for muscularity) including 22 type traits, was introduced in Hungary in 1986. The qualification system was developed for the type classification of cows and bulls. The investigation was carried out using 207 cows and 324 sire candidate young bulls from two Limousine seedstock herds (A, B).

The aim of the investigation was to answer the following question: How can the type classification system be modified or simplified?

For the statistical analysis a multiple regression analysis (backward stepwise) was used. The results suggest that the type classification system should be modified due to the fundamental importance of indirect selection. The estimated linear-type traits characterised the final summit of the phenotypic score by a multiple correlation coefficient (r = 0.999) and by a low deviation (0.369) in cows. After 13 steps, only 9 of the original 22 type traits were incorporated in the regression equation. The value of the multiple correlation coefficients did not change substantially after the 13th step of the analysis (r = 0.991). The results for bulls were similar to the results for cows (step 0: r = 0.997; step 12: r =0.988). In this case, after 12 steps only 10 of the original 22 type traits were incorporated in the regression equation. The results of multiple regression analysis indicated that it is possible to reduce the number of type traits.

Key words: Limousine, cows, bulls, type classification, type traits

Introduction

Among professionals there is no doubt that the connection between phenotype and expected production can be established more easily in beef cattle than in dairy types. On the one hand the ability of the animals to produce beef can be estimated visually. On the other hand, the heritability values of phenotypic characters are relatively high (h^2 = 0.4–0.6). A correlation coefficient of at least r = 0.70 could be calculated by the estimation of muscularity, between living and slaughtered traits. This supports the application of type classification in practice (Korchma, 1986; Journaux, 1994). Therefore, the results of type classification have a particular and definite role – the determination of efficient selection – in breeding programmes for beef cattle.

The Association of Limousine Breeders has developed a qualification system for the type classification of cows, which includes the following factors: calving interval, 205-day weaning weight of calves, and the total number of points in the phenotypic score of suckling cows. In the selection index

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(combined index) these traits had weights of 50, 35 and 15%, respectively (Balika and Bíró, 1993a).

Sire candidates of 12 months of age were officially qualified at the end of the performance test (PT). In the sire qualifying index, live weights adjusted to 205 and 365 days of age were given weights of 20–40% (Balika and Bíró, 1993a). The corresponding weights for the type scores (value of utility, length and width measures, muscularity) was 10%.

Among the multiple regression methods factor analysis was used in type classification in earlier years by Sieber et al. (1988) in Holstein-Friesian cows, Márton et al. (1988) in Hereford cows and Vági (1992) in Limousine cows. Tőzsér et al. (1997) carried out a study on the results of type classifications including 327 Charolais sire candidates. The utility value traits showed considerable heterogeneity. This group of traits consists of six part traits: shoulder stability, the strength of the back and rump (10.7%) and the leg structure and skeleton (10.4%) factors could be clearly separated.

In relation to the type classification systems applied in national and international practice (based on the works of Korchma, 1986; Dubois and Huneault, 1990; Anon., 1990; Boonen, 1991; Rehben, 1992; Balika and Bíró, 1993b; Anon., 1997) the following factors should be considered:

- The number of groups of type traits differs from country to country: Hungary and Belgium, 4; France, 3; Canada, 0; the number of type traits in the type classification systems in these countries is Hungary, 22; Belgium, 20; France, 14; Canada, 4.
- The scoring structure used in the various countries involves 1–10 points in Hungary and France; 1–50 or 1–25 points in Belgium; and 1–9 points in Canada and Germany.
- The number of phenotypic points is calculated using different methods.
- The condition of the cattle examined is considered to be additional information.
- All previous experience should be taken into consideration to improve the type classification system for Hungarian beef cattle breeding, together with an improvement in performance test evaluation. The aim of the investigation was to answer the following question: How can the Limousine type classification system be modified or simplified?

Materials and methods

Phenotypic scores were estimated for animals in two Limousine seedstock herds (A: n = 60; B: n = 147; $\sum n$ = 207 cows, and A: n = 47; B: n = 277, $\sum n$ n = 324 bulls, respectively). The official type classifications for the cows was carried out at the age of 4–5 years after the second calving. Sire candidates of 12 months old were officially qualified at the end of the performance test.

In the type classification 22 type traits, divided into 4 groups, were estimated by the following methods:

- utility score (height at withers, chest depth, shoulder stability, strength of back and rump, leg structure, skeleton)
- score for length (length of the body, length of the back, length of the loin, length of the rump)

- score for width (width at withers, chest width, loin width, rump width I, rump width II, rump width III)
- score for muscularity (muscularity of breast, muscularity of shoulder, muscularity of back, muscularity of loin, length of rump, muscularity of rump).

Balika and Bíró (1993b) changed the type classification system for Limousine cows and bulls, modifying the scale to 1–9 points by omitting the rump width III and separating the width at the withers from the back width. In this modified classification system 1–3 points indicated undesirable stature and 4–6 points excellent stature. In the present investigation the rump width III was retained. In the statistical procedure multiple regression analysis (backward stepwise) was used.

Results and discussion

The phenotypic scores for the Limousine cows and bulls evaluated (mean and deviation values) are summarized in Table 1. By estimating the results of all 4 groups of traits for the cows it could be established that "good" (> 61 points) standard performance was only achieved in the case of utility score.

Table 1
Type classification scores for Limousine cows and bulls

Traits	Cows (n=207) Mean ± S.D.	Bulls (n=324) Mean ± S.D.
Height at withers	5.15±1.01	5.30±1.15
Chest depth	5.68±1.00	5.77±1.11
Shoulder stability	6.02±1.02	6.32±0.86
Strength of back and rump	6.05±1.02	6.38±0.87
Leg structure	6.01±0.80	5.86±0.96
Skeleton	6.77±1.05	6.71±0.95
Utility score	**66.08±8.32**	**67.31±8.51**
Length of the body	5.29±0.89	5.62±1.18
Length of the back	5.41±0.99	5.60±1.30
Length of the loin	4.95±1.00	5.60±1.15
Length of the rump	5.04±0.99	5.31±1.20
Score for length	**57.51±9.32**	**61.45±12.46**
Width at withers	4.75±0.99	5.16±1.04
Chest width	4.85±0.88	5.47±1.01
Loin width	5.21±0.92	5.85±1.06
Rump width I	5.46±0.95	5.70±1.08
Rump width II	5.21±1.04	5.31±1.05
Rump width III	4.85±1.03	5.07±1.08
Score for width	**56.17±9.50**	**60.30±10.88**
Muscularity of breast	4.59±1.04	5.24±1.16
Muscularity of shoulder	4.47±1.10	5.35±1.26
Muscularity of back	4.92±1.02	5.88±1.27
Muscularity of loin	4.96±1.17	5.52±1.24
Length of rump	5.04±1.16	5.51±1.30
Muscularity of rump	4.68±1.13	5.27±1.26
Score for muscularity	**53.10±11.31**	**60.68±13.06**
Total phenotypic score	**58.22±8.87**	**62.44±10.64**

For the length, width and muscularity scores only average (51–60 points) standard performance values were recorded. The total phenotypic score was also in the average category. The results in all 4 groups of type traits for the bulls were the following: utility score (67.31±8.51), score for length (61.45±12.46), score for width (60.30±10.88) and score for muscularity (60.68±13.06).

These results support the need to modify the type classification system due to the fundamental importance of indirect selection in Limousine cows and bulls.

The results of multiple regression analysis (backward stepwise) are shown in Table 2.

Table 2
Results of the multiple regression analysis (backward stepwise)

Dependent variable (y)	Independent variables (x_1–x_{22})	Cows (n=207)		Bulls (n=324)	
		Regression coefficients, step 0 (b_1–b_{22})	Regression coefficients, step 13 (b_1–b_9)	Regression coefficients, step 0 (b_1–b_{22})	Regression coefficients, step 12 (b_1–b_{10})
	Height at withers	0.052	–	0.053	–
	Chest depth	0.052	0.110	0.049	–
	Shoulder stability	0.062	0.099	0.039	0.081
	Strength of back and rump	0.053	–	0.039	–
	Leg structure	0.046	0.086	0.041	–
	Skeleton	0.050	–	0.041	0.070
	Length of the body	0.068	–	0.075	–
	Length of the back	0.070	0.132	0.079	0.121
	Length of the loin	0.079	0.145	0.071	0.128
Total	Length of the rump	0.080	0.123	0.074	0.130
phenotypic	Width at withers	0.047	–	0.048	0.131
score	Chest width	0.059	–	0.046	0.124
	Loin width	0.048	–	0.047	–
	Rump width I	0.054	–	0.047	–
	Rump width II	0.048	–	0.048	–
	Rump width III	0.052	0.181	0.048	–
	Muscularity of breast	0.048	–	0.051	–
	Muscularity of shoulder	0.073	0.220	0.054	0.126
	Muscularity of back	0.040	–	0.055	0.122
	Muscularity of loin	0.064	–	0.054	–
	Length of rump	0.056	–	0.055	–
	Muscularity of rump	0.058	0.138	0.053	0.107
Parameters	Constant	0.2221	3.1383	0.2659	2.0803
of regression	MCC	0.999***	0.991***	0.997***	0.988***
equation	SEE	0.369	1.143	0.891	1.641

***= Statistically significant effects at P<0.001; MCC: Multiple correlation coefficient; SEE: Standard error of estimation

In the first step of the analysis all the estimated type traits (x_{1-22}) were introduced into the regression equation. The estimated linear type traits characterised the whole of the phenotypic score by a multiple correlation coefficient (r = 0.999) and by low deviation (0.369) in the cows. The regression coefficients ranged from b = 0.046 (leg structure) to b = 0.080 (length of the rump) ($P < 0.001$). During the analysis the computer program omitted, step by step, traits which had no statistically important effects on the final value of the phenotypic score. After 13 steps only 9 of the 22 type traits were retained in the regression equation. These were as follows: chest depth, shoulder stability, leg structure, length of the back, length of the loin, length of the rump, rump width III, muscularity of shoulder, muscularity of rump. The regression coefficients ranged between b = 0.086–0.220, ($P < 0.001$) in this case. The value of the multiple correlation coefficients did not change substantially after the 13th step of the analysis (r = 0.991). Consequently, if 9 type traits were introduced into the equation they gave a similar characterisation of the final score of the type classification, with a somewhat larger deviation.

The results for the bulls were very similar to those for the cows (step 0, r = 0.997; step 12, r = 0.988). After 12 steps only 10 of the 22 type traits were retained in the regression equation. These were as follows: shoulder stability, skeleton, length of the back, length of the loin, length of the rump, width at withers, chest width, muscularity of shoulder, muscularity of back, muscularity of rump.

Based on this estimation it is possible to reduce the number of type traits in the Limousine type classification systems for both cows and bulls.

The results are in agreement with previous evaluations of the type classification in Charolais young breeding bulls (Tőzsér et al., 1997). This suggests some modifications in the type classification system; for instance, rump width III was introduced as a "suggested" variable based on the results of regression analysis. However, due to its complicated estimation, it would be useful to substitute rump width I or rump width II for rump width III.

Conclusions

The results of multiple regression analysis (backward stepwise) indicate that it is possible to reduce the number of type traits used in type classification systems for Limousine cows and sire candidates.

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Short communication

RELATIONSHIP BETWEEN SEED YIELD AND SEED CHEMICAL COMPOSITION IN KABULI CHICKPEA UNDER SEMIARID MEDITERRANEAN CONDITIONS

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The chemical composition of chickpea seeds is modified by cultivar and environment. This study was conducted to investigate the relationships between the seed protein, lipid, starch and sugar concentrations of kabuli chickpea (*Cicer arietinum* L.) and the seed yield under semiarid Mediterranean conditions. Four kabuli chickpea cultivars (3 improved and one local) were grown under rainfed conditions in northern Jordan for two growing seasons. Significant differences were noted between the cultivars for each character except fructose concentration. Seed yield, and the seed protein, lipid, glucose and fructose concentrations were significantly affected by the growing season. Significant year × cultivar interactions were noted for seed yield, and for seed protein, lipid and glucose concentrations. A negative correlation was noted between seed yield and the seed protein and glucose concentrations. Positive, non-significant correlations were found between seed yield, and the seed fructose and starch concentrations. Seeds of the local cultivar had the highest protein, glucose and sucrose concentrations among the cultivars, even though it produced the lowest seed yield, whereas seeds of the cultivar Jubeiha-3 had the lowest glucose and starch concentrations among the cultivars, even though it produced the highest seed yield. These results indicate that increases in seed yield as the result of favourable weather conditions or the cultivation of more productive cultivars tend to reduce the nutritional quality of chickpea seeds.

Key words: *Cicer arietinum* L., lipid, protein, starch, sugar, yield

Introduction

Chickpea is the world's third most important grain legume after beans and peas (FAO, 1993). It is grown on the Indian subcontinent, in the Mediterranean Basin, and in East Africa, Mexico and Australia. In the Mediterranean countries, kabuli chickpea is considered a very important legume crop. It is utilized in many forms, e.g. canned, roasted, boiled and puffed. It is also used in preparing dishes such as Falafel and Homos biteiheneh, which are very popular and consumed on a daily basis. Chickpeas and other food legumes contribute significant amounts of protein, carbohydrate, vitamins and minerals to the diets of people living in the Mediterranean region (Bahl, 1990; Singh et al., 1993).

From the economical and nutritional standpoints, increasing the quantity of food (e.g. yield) and improving the quality (e.g. protein) of these foods is

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beneficial. Protein improvement in legumes has been hampered because breeding programmes have produced cultivars primarily for high yield, and correlations between yield and seed protein have generally been negative (Henry et al., 1995).

Environmental conditions exert significant influences on the chemical composition of legumes (Al-Karaki and Ereifej, 1997; Singh et al., 1990), and significant genetic variations in the chemical composition of legume seeds have been reported (Al-Karaki and Ereifej, 1997; Bajaj, 1975; Ereifej and Al-Karaki, 1996). Breeding programmes are designed to search for high-yielding chickpea cultivars to meet the increasing demand for chickpea seeds. The chemical composition of the seeds of improved or released chickpea cultivars is not emphasized. The objective of the present work was to study the relationships between the seed protein, lipid, starch and sugar concentrations of four kabuli chickpea cultivars, three improved and one local, grown under rainfed conditions in northern Jordan for two growing seasons.

Materials and methods

The chickpea material consisted of 3 improved cultivars (Jubeiha-1, Jubeiha-2 and Jubeiha-3) and one local variety. Seeds of these cultivars were provided by the National Center for Agricultural Research and Technology Transfer (NCARTT), Amman, Jordan. The experiments were conducted under rainfed conditions in the 1994/95 and 1995/96 (hereafter called 1995 and 1996, respectively) growing seasons at Maru Research Station (northern Jordan), located at 34°40'N at an altitude of 590 m. The soil type was silty clay (fine, montmorillonitic, thermic, Entic Chromoxeret) with low levels of organic matter (1.2%) and pH 7.9. This location has a typical Mediterranean climate. The long-term average rainfall is 370 mm. Phosphorus fertilization was applied at a rate of 30 kg P ha^{-1} at the time of sowing. The experimental design was a randomized complete block with three replications. Plots were 1.4 m × 4 m with 4 rows spaced 0.35 m apart and 10 cm between plants. The seeds received no rhizobial inoculation. Weeds were controlled by hand.

At seed maturity, pods from plants in the two middle rows of each plot were harvested manually, sun dried for two weeks, and threshed manually. The seeds were weighed for the determination of seed yield.

Seed samples (100 g) from each replication were oven-dried, weighed, ground to pass a 0.5 mm screen using a Cycotec mill and prepared for chemical analysis. Seed protein (N × 6.25) and crude fat (ether extract) concentrations were determined according to AOAC (1984). Glucose, sucrose and fructose concentrations were determined using the phenol-sulphuric reaction according to the method described by Dubois et al. (1956). Seed starch was determined colorimetrically after extraction with 80% ethanol and solubilizing with dilute perchloric acid (McCready et al., 1950).

The data were subjected to analysis of variance (main effects and interaction) and least significant difference (LSD) values were used to evaluate the significance of differences between means. Simple correlations were computed between different chemical composition parameters and seed yield.

Results and discussion

Annual rainfall was 388 and 335 mm for 1995 and 1996, respectively. Rainfall was greater, started earlier, and finished earlier in 1995 than in 1996 (see Al-Karaki and Ereifej, 1999). The late start and the low total rainfall in 1996 were most likely responsible for the lower seed yields compared to 1995.

Analysis of variance indicated that the growing season (year) significantly influenced all traits except sucrose and starch concentrations (Table 1). There were statistically significant variations between the cultivars for all traits except fructose concentration. Significant year × cultivar interactions occurred for seed yield, and for seed protein, lipid and glucose concentrations (Table 1). Berke et al. (1992) reported significant genetic-environmental interactions, indicating that cultivars responded differently across environments.

Averaged over the cultivars, both years differed markedly in seed yield, and in seed protein, lipid, glucose and fructose concentrations (Table 2). Chickpeas grown in the 1995 season (388 mm rainfall) exhibited lower protein and lipid concentrations than those grown in the 1996 season (335 mm rainfall). These facts may indicate an inverse relationship between the productivity of the season and the protein and lipid contents, but the lowest concentrations of glucose and fructose occurred in 1996. Singh et al. (1993) reported that the chemical composition of chickpea seeds varied under different environmental conditions. It is suggested that environmental factors (rainfall) might have affected the transport of assimilates to the seed, thus affecting the chemical composition of the chickpea seeds.

Genotypic variation was relatively large for seed yield, and for seed protein, lipid, glucose and sucrose concentrations, and relatively low for starch (Table 2). The correlation coefficients between seed yield and different chemical composition parameters are presented in Table 3. Considerable portions of the variation in the protein, lipid, starch and sugar concentrations were attributable to genotypic differences in seed yield, and concentrations of seed protein and glucose usually decreased as the seed yield increased. Al-Karaki and Ereifej (1999) reported that the protein and fructose concentrations of pea (*Pisum sativum* L.) seeds decreased as seed yields increased.

Table 1
Probabilities of significance for seed yield and chemical composition parameters in chickpea

Trait	Year (Y)	Cultivar (C)	Y × C
Seed yield	*	**	**
Protein	*	**	**
Lipid	**	**	**
Glucose	**	**	**
Fructose	**	NS	NS
Sucrose	NS	**	NS
Starch	NS	*	NS

*, ** : significant at $P<0.05$ and $P<0.01$, respectively; NS : not significant

Table 2
Year, cultivar and year × cultivar effects on seed yield and chemical composition in chickpea

	Seed yield (kg ha^{-1})	Protein	Lipid	Glucose	Fructose	Sucrose	Starch
				(mg g^{-1} DM)			
Year							
1995	1014	181	66	41	57	31	314
1996	812	184	70	35	44	29	313
SE†	*40*	*2*	*1*	*2*	*3*	*2*	*6*
Cultivar							
Jubeiha-1	961	174	66	38	48	26	323
Jubeiha-2	885	174	66	34	44	24	326
Jubeiha-3	1159	187	71	34	55	31	298
Local cultivar	646	194	69	47	53	41	299
$LSD_{0.05}$	*120*	*5*	*2*	*5*	*8*	*4*	*20*
1995							
Jubeiha-1	1005	167	65	41	52	26	328
Jubeiha-2	999	183	65	38	46	26	318
Jubeiha-3	1355	177	64	33	60	28	300
Local cultivar	695	197	68	54	69	47	296
1996							
Jubeiha-1	916	180	67	35	45	25	318
Jubeiha-2	770	166	66	29	43	22	33
Jubeiha-3	963	197	78	35	50	34	298
Local cultivar	597	191	70	39	37	35	303
$LSD_{0.05}$	*169*	*7*	*3*	*7*	*11*	*5*	*28*

†: SE = standard error.

Seed yield and the seed fructose and starch concentrations were positively correlated, whereas seed yield and the protein, lipid, glucose and sucrose concentrations were negatively correlated (Table 3). Factors other than seed yield appeared to be important in determining the concentrations of protein, lipid, glucose and sucrose. Yield-independent genotypic variation could be demonstrated best by comparing cultivar Jubeiha-3 and the local cultivar. Seeds of the local cultivar had the highest protein, glucose and sucrose concentrations among the cultivars, even though it produced the lowest seed yield, whereas seeds of Jubeiha-3 had the lowest glucose and starch concentrations among the cultivars, even though it produced the highest seed yield. The chemical composition of chickpea seeds varied according to variety and climatic conditions (Singh et al., 1990; 1993).

Table 3
Correlation coefficients (r) of seed yield with chemical composition parameters (combined data, n=4)

	Seed yield
Protein concentration	–0.43*
Lipid concentration	–0.24
Glucose concentration	–0.47*
Fructose concentration	0.25
Sucrose concentration	–0.39
Starch concentration	0.23

* : Significant at $P<0.05$

In conclusion, increases in seed yield as the result of favourable weather conditions or the cultivation of more productive cultivars tended to reduce the nutritional quality of chickpea seeds.

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Review

AN ANALYSIS OF THE HUNGARIAN FOOD MARKET

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In this paper the market shares of 10 items on the Hungarian food market are analysed. The volume of the overall consumption of these items is approximately 50% of the whole food market. The methodology used in this paper is based on a new linear model, taking into account dynamic changes in the market. The objective of the paper is to suggest a method for forecasting the future behaviour of consumer groups. The main problem is that due to the changing tastes of consumers the forecasting power of static models is restricted. In the present model this phenomenon is described by special variables.

Key words: operations research, modelling, statistical analysis, Almost Ideal Demand System, family statistics

Introduction

In Hungary the food industry plays a decisive role in satisfying household demands and has a large positive export-import balance (Hajduné Balogh, 1994). The efficiency of food exports is better than that of the majority of other products in the Hungarian economy, which is why food industrial exports are of vital importance from the viewpoint of the external trade balance of the national economy (Szabó, 1999). The dynamic changes in the foreign markets targeted by the Hungarian food industry (collapse of COMECON, emergence of a single European market) gave a new impulse to the reconsideration of the competitiveness of the Hungarian agri-food sector. Import liberalization and increasing competition on foreign markets underline the importance of the home market. Contrary to previous expectations, the Hungarian foreign trade balance with EU member states drastically worsened after the EU – Hungarian Foreign Trade Treaty (Fig. 1). The declining income of Hungarian citizens, which stagnated in the mid-nineties, decreased the domestic purchasing power and total demand (Fig. 2).

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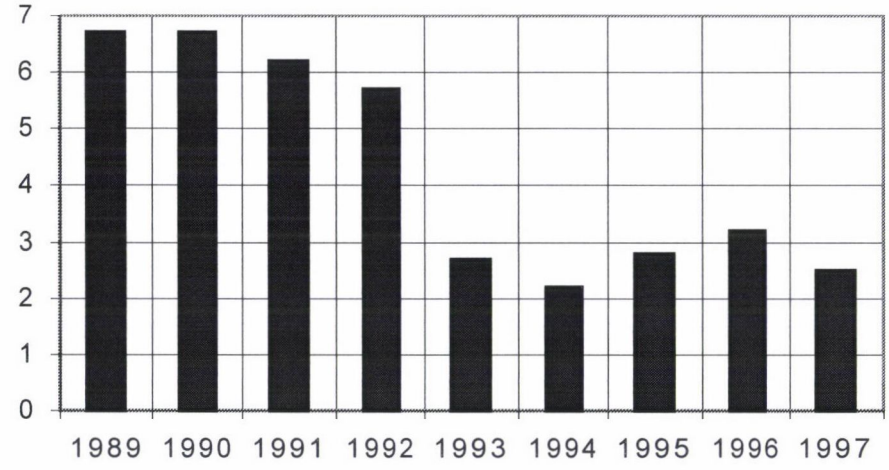

Fig. 1. The export-import ratio in Hungary-EU agricultural trade
(Source : KSH Statistical Yearbooks, 1989–1999)

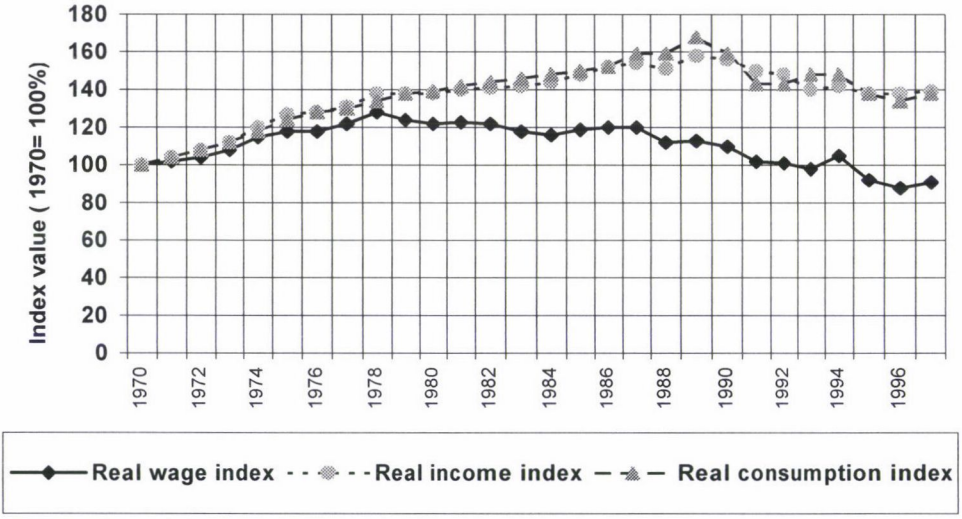

Fig. 2. Income and consumption of Hungarian consumers (1970–1997)
(Source: KSH Statistical Yearbooks, 1970–1999)

The analysis of internationally competitive branches of the food economy in some European states underlines the fact that the security of internal markets is a necessary precondition for export success. The poor health status of Hungarian citizens is another fact which underlines the importance of studies dealing with the buying habits and nutrition of Hungarian consumers.

In conclusion it can be stated that the survey of food consumption and the forecast of the food market is of great interest. Forecasting is relatively easy only when the market is stable and no structural changes are taking place in it. One popular method is the application of the Almost Ideal Demand System (AIDS) model (Deaton and Muellbauer, 1980).

The key data required by the AIDS model are the price elasticities of the commodities. Elasticities are usually determined from the price and consumption data of longer periods. Reliable results can only be obtained if the basic conditions and the structure of the market are unchanged, but this assumption is very rarely true. Therefore, it can be observed that the absolute value of the elasticities is often less than the standard error of the calculation (Sasaki, 1990; Rickertsen, 1998). This means that even the sign of the elasticity cannot be determined. Thus the reliability of any forecast using these data is questionable.

In this paper a new linear model is suggested which contains, among other things, a group of variables for the description of changes in the tastes of consumers.

The Linear Model

It is assumed that changes in the market shares are caused by three factors: (a) changes in prices, (b) changes in consumer incomes and (c) changes in consumer tastes. It is also assumed that the last factor has a trend and that the changes are linear in time. A certain year, say year 0, is fixed and all changes are relative to that year.

Factor (a), i.e. a change in price, is assumed to have a direct influence only on the appropriate item. Indirectly, of course, it affects the market shares of all other goods. Moreover, it is assumed that factors (b) and (c) may produce direct interactions among the goods, i.e. some commodity must give a part of its market share to other goods. For example, the increasing use of vegetable oil instead of lard is a phenomenon observable in Hungary. In this context, each year lard gives an increasing part of its earlier market share to vegetable oil. Groups of goods are formed according to their nutritional roles. Direct interaction is assumed to be possible only within groups.

The variables and indices used in the model are as follows:

n the number of different goods
i the index of the item
m the number of years to be compared to year 0
k the index of the year
δ_{ij} the yearly increase in the market share of item i at the expense of item j
ρ_{ij} the market share of item j gained from the share of item i as a result of the changes in consumer income
Φ_i the loss/gain of the market share of item i caused by a change in its price

p the number of groups
l the index of the group
G_l group l
$G(i)$ the group containing item i.

For the sake of convenience two notations will be used for the groups. If the group containing item i is required it will be denoted $G(i)$. If only the groups are to be listed they will be denoted by G_l ($l=1,...,p$).

The input data of the model are the following:

w_{ik} market share of item i in year k
y_k consumer income in year k
p_{ik} price of item i in year k.

In year k the market share of item i gained at the expense of item j due to changes in consumer tastes is:

$$k\delta_{ij}$$

assuming that the change happens k years after the initial year, as the relationship is assumed to be linear. From this it follows that

$$k\delta_{ij}$$

is the loss in the market share of item j in year k. In calculating the effect of price changes, consumer incomes must be taken into consideration. Thus, the effect of the change in item i on the market share is

$$(p_{ik} - p_{i0})\frac{Y_k}{Y_0}\Phi_i$$

To be consistent, the change in the income is measured relative to the income in year 0. Thus, this effect in the case of item i can be given in the form

$$\frac{Y_k - Y_0}{Y_0}\sum_{j=1}^{n}\rho_{ij}.$$

This follows from the assumption that this type of change causes a direct interaction among the market shares.

Hence, the market share of item i in year k can be determined by the equation

$$w_{ik} = w_{i0} + k\sum_{j\in G(i)}(\delta_{ij} - \delta_{ji}) + (p_{ik} - p_{i0})\frac{Y_k}{Y_0}\Phi_i + \frac{Y_k - Y_0}{Y_0}\sum_{j\in G(i)}\rho_{ij} \quad (1)$$

There are mn equations of type (1) and there are

$$N = \sum_{l=1}^{p}|G_l|^2$$

variables, where $|G_l|$ denotes the number of elements of G_l. Thus, assuming that the above equations are independent, the system is solvable only if

$$mn \leq N \; . \quad (2)$$

If (2) is satisfied with strict inequality, i.e. the number of variables exceeds the number of equations, then there exist an infinite number of solutions. Thus a principle is needed for choosing an appropriate one for forecasting. On the other hand, if (2) is not satisfied, then there exist only approximate solutions. One such solution can be determined by the least squares method.

Numerical results

In the actual model three groups were used as follows:

Group 1. Pork, poultry, cheese, milk, eggs.
Group 2. Sugar, lard, vegetable oil.
Group 3. Potatoes, bread.

The calculations were based on the data of the Hungarian Central Bureau of Statistics (1993–1996). This series of annals divides the families into 10 groups (deciles) according to the incomes of the families. Three consecutive groups, more precisely groups 3, 4, and 5, were chosen as the subject of the investigation. The HCBS statistics provide us with the market shares of each item for each income group in each year. Although there are earlier issues of the series the years 1993–1996 were chosen for two reasons. First of all, large-scale economic and social changes took place in Hungary after 1989, but the basic structure of the economy has not changed much since 1993. The HCBS series does not have an issue for 1992. If the 1991 issue is used the data system will not be continuous in time. As the basis for comparison the market share of group 4 in 1993 was selected. It is important to emphasize again that for the income changes in the particular model, not only on a yearly basis but in each year, three different income levels were taken into consideration.

The constraints and variables of the model are as follows. The number of equations of type (1) is 110, since income groups 3 and 5 have 10 equations for the 10 items in each year, making twice 40 equations, and income group 4 has 3 times 10 equations for years 1994, 1995 and 1996, as 1993 was the basis of comparison. The number of variables of types δ and ρ in the three groups of goods is 20, 6 and 2, respectively. For each item there is one variable of type Φ. Thus the total number of variables is 66. This means that the equation system is overdetermined. The least squares method gives the best approximate solution.

In the calculations both the prices and the incomes were given in real (deflated) Hungarian Forints, adjusted for inflation. The actual and calculated market shares of the deciles (income groups) are given in Tables 1, 2 and 3. The values of variables of types δ, ρ and Φ can be found in Tables 4, 5 and 6, respectively.

The errors of the calculated market shares are between –1.6% and 1%, except for one error, which is 2.65%. The results fit well to the real data (R^2=0.9942).

In 1995 a Giffen effect was observable in the case of potatoes. Although real incomes decreased and the real potato price increased, the total consumption increased in each income level. This explains the great negative sensitivity of the market share of potatoes relative to income (see Table 5).

Table 1
Market shares from the total consumption of family income decile 3

Item	Actual shares				Calculated shares			
	1993	1994	1995	1996	1993	1994	1995	1996
Pork	22344	24662	23054	24448	24988	24479	23551	22852
Poultry	14183	13633	15615	14302	14148	14419	14742	15085
Milk	15224	14229	13819	13798	13702	14207	14364	13893
Cheese	3164	2415	2354	2966	2492	2713	2840	2953
Eggs	6441	6798	5958	6941	6765	7070	6577	6683
Sugar	6124	6295	4708	5477	6509	6410	5330	5480
Lard	3866	3519	3469	3120	3409	3319	3549	3283
Oil	2954	3113	3954	3782	3151	3109	4030	3944
Potatoes	5750	6226	8769	4934	6030	6514	8498	4948
Bread	19928	19111	18300	20231	19879	19166	18359	20148

$\times 10^{-5}$

Only meat, i.e. pork and poultry, may lose part of its market share with an increase in prices. This means that the consumption of these two products is above the necessary level and the consumption may decrease. All other products will be consumed at least at the same level.

The final result of the change in consumer tastes is that eggs, cheese and poultry have gained market shares from milk and pork. Vegetable oil has also gained a market from lard. An increase in income causes similar effects. Again lard is the great loser.

Table 2
Market shares from the total consumption of family income decile 4

Item	Actual shares				Calculated shares		
	1993	1994	1995	1996	1994	1995	1996
Pork	25397	24359	23201	22739	24913	23982	23240
Poultry	14449	14845	15862	15177	14696	15063	15381
Milk	13237	14933	13494	13788	13858	13918	13299
Cheese	2529	3356	2884	3205	2763	2883	2985
Eggs	6624	6970	6127	7218	7034	6386	6549
Sugar	6650	5692	5359	6607	6531	5615	5494
Lard	3361	3338	3354	3240	3257	3493	3230
Oil	3190	2833	4100	4370	3134	4196	4093
Potatoes	5843	6342	8645	4639	6488	8682	4657
Bread	18721	17331	16974	19018	17648	16621	18635

$\times 10^{-5}$

Table 3
Market shares of family income decile 5

Item	Actual shares				Calculated shares			
	1993	1994	1995	1996	1993	1994	1995	1996
Pork	24785	25474	25310	23484	25777	25301	24388	23613
Poultry	14253	15456	15639	16046	14728	15009	15356	15635
Milk	13989	13524	12959	13843	12805	13307	13406	13068
Cheese	2801	2897	3130	3500	2563	2791	2915	3024
Eggs	6736	6831	6213	6905	6493	6821	6295	642
Sugar	6776	6598	5595	5857	6781	6686	5648	5839
Lard	3219	3395	3751	3305	3316	3219	3445	3200
Oil	3204	3166	3636	4495	3226	3183	4086	3929
Potatoes	5751	5986	7995	4705	5670	6200	8052	4691
Bread	18487	16674	15772	17861	17646	16740	16062	18300

$\times 10^{-5}$

Table 4
Changes in market shares caused by changes in consumer tastes(δ's)

	Pork	Poultry	Cheese	Milk	Eggs	Sugar	Lard	Oil	Potatoes	Bread
Pork	0	0	0	0	0	0	0	0	0	0
Poultry	0	0	0	0	672	0	0	0	0	0
Cheese	0	182	0	0	0	0	0	0	0	0
Milk	0	0	0	0	0	0	0	0	0	0
Eggs	438	0	0	553	0	0	0	0	0	0
Sugar	0	0	0	0	0	0	0	32	0	0
Lard	0	0	0	0	0	0	0	0	0	0
Oil	0	0	0	0	0	0	56	0	0	0
Potatoes	0	0	0	0	0	0	0	0	0	0
Bread	0	0	0	0	0	0	0	0	215	0

$\times 10^{-5}$

Table 5
Changes in market shares caused by change in income (ρ's)

	Pork	Poultry	Cheese	Milk	Eggs	Sugar	Lard	Oil	Potatoes	Bread
Pork	0	4003	0	0	0	0	0	0	0	0
Poultry	2942	0	0	0	0	0	0	0	0	0
Cheese	361	0	0	0	0	0	0	0	0	0
Milk	–4554	0	0	0	0	0	0	0	0	0
Eggs	–1380	0	0	0	0	0	0	0	0	0
Sugar	0	0	0	0	0	0	1379	0	0	0
Lard	0	0	0	0	0	–472	0	0	0	0
Oil	0	0	0	0	0	382	0	0	0	0
Potatoes	0	0	0	0	0	0	0	0	0	–1829
Bread	0	0	0	0	0	0	0	0	–11333	0

$\times 10^{-5}$

Table 6
Changes in market shares caused by changes in prices (Φ's)

Pork	Poultry	Cheese	Milk	Eggs	Sugar	Lard	Oil	Potatoes	Bread
–1	–15	373	2	579	75	18	103	212	678

$\times 10^{-5}$

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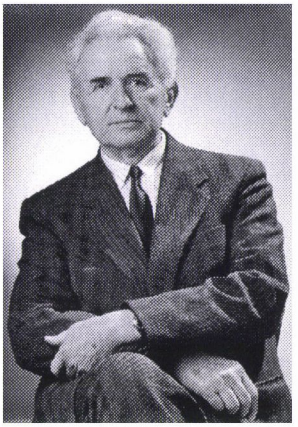
Obituary

IN MEMORIAM
PROF. SLAVKO BOROJEVIĆ
(1919–1999)



Prof. Slavko Borojević died in Novi Sad on 19 September 1999.

Born on 21 November 1919 in the village of Knezovljani, Kostajnica, Croatia, Slavko Borojević finished senior high school in 1939 in Sisak and was admitted to the Faculty of Agriculture and Forestry of the University of Zagreb in the same year.

His academic career was interrupted by the war. He joined the National Liberation Army from 1941 to 1945.

In October 1945 he re-enrolled in the Faculty of Agriculture and Forestry of Zagreb University and graduated in July 1947. In June 1948 he was appointed assistant at the Department of Genetics of the same Faculty, where he received a Ph.D. degree in June 1953. The title of his Ph.D. thesis was "Heterosis in rye crosses between domestic populations and selected varieties".

In 1950/51 he spent a year studying genetics at the Department of Genetics in Cold Springs Harbor and at the Department of Agronomy and Genetics, University of Minnesota in St. Paul, U.S.A.

In 1956 he was promoted to the post of assistant professor at the Faculty of Agriculture and Forestry in Zagreb and in 1957 he transferred to the Faculty of Agriculture in Novi Sad where he was appointed associate professor in the Department of Genetics and Plant Breeding.

In 1960/61 he studied wheat breeding at the Institute of Plant Breeding in Bologna and at the Institute of Genetics in Rome, Italy.

He became full professor in 1962 and taught genetics in this capacity until his retirement. He also supervised postgraduate students in genetics and plant breeding and taught the courses Theory of Plant Breeding and Introduction to Research Methodology.

In 1969/1970 he spent a year at the Institute of Agriculture in Cairo, Egypt, as FAO/IAEA expert on plant breeding.

From 1957, when he came to Novi Sad, till 1975, he was concurrently a lecturer and head of the Wheat Department of the Institute of Agricultural Research in Novi Sad.

From 1958 to 1976 he was editor-in-chief of the monthly journal "Contemporary Agriculture".

From 1973 to 1978 he was president of the Yugoslav Society of Genetics. He was also a member of the Executive Board of the International Wheat Genetics Symposium and the Board of Representatives of the International Congress of Genetics, Dean of the Agricultural Faculty (1960–1962), Rector of the University of Novi Sad (1974–1976), President of the Association of Universities of Yugoslavia (1974–1976), a member of the Board of the European Association of Plant Breeders (1971–1974), a member of the Council of the International Congress of Genetics (1973–1978), a member of the International Organizing Committee of the Wheat Genetics Symposium (1973–1978), Secretary of the Department for Natural Sciences of the Academy of Sciences and Arts of the Vojvodina Province (1980–1984), President of the Academy of Sciences and Arts of the Vojvodina Province (1984–1988) and a member of the Editorial Board of the German scientific journal Plant Breeding (1980–1994).

In 1970, Prof. Slavko Borojević was appointed a foreign member of the All-Union Academy of Agricultural Sciences in Moscow for achievements in genetics and plant breeding. In 1991 he became a full member of the Serbian Academy of Sciences and Arts, in 1986 an honorary member of the American Society of Agronomists and in 1999 a member of the Academy of Agriculture of Slovakia.

In 1977 he received an honorary doctorate from the University of Agricultural Sciences in Gödöllő, Hungary.

As a result of his research work, Slavko Borojević published 140 research and technical papers, 30 of these abroad. He wrote seven books, of which three had a second edition. He was also the co-author of several monographs. His most important books are: Genetics (with his wife, Katarina Borojević), published in 1971 and 1976, Methodology of Experimental Research Work, published in 1974 and 1992, Principles and Methods of Plant Breeding, published in 1981 and 1992, Genetic and Technological Changes that Transformed Plant Breeding, 1983. The book Principles and Methods of Plant Breeding was translated into Russian ("Kolos", Moscow, 1984) and English ("Elsevier", Amsterdam, 1990).

Prof. Borojević also published his war diary, entitled "On the glorious path", in which he kept records of the crucial battles of the liberation war, the 4th and 5th enemy offensives.

Professor Borojević made a significant contribution at the national and international level in several fields of work: genetic study of quantitative traits, theory of plant breeding, development of models of high-yielding wheat cultivars, development of new wheat cultivars and utilization of their genetic yield potentials.

He was the principal breeder of 50 winter wheat and 8 spring wheat cultivars and co-breeder of 41 winter wheat cultivars, 5 spring wheat cultivars and one winter triticale cultivar. Many of these cultivars were commercially grown on considerable acreage in Yugoslavia, while some of them, notably Sava, were successfully grown in the Czech Republic, Hungary, Slovakia, Romania, etc. The results achieved in wheat breeding made Professor Borojević one of the most successful wheat breeders in Europe. Breeders in Yugoslavia and abroad consider him as the founder of the "Yugoslav school of plant breeding".

As a teacher, Professor Borojević taught the course Genetics to graduate students and the courses Introduction to the Methodology of Research Work, Cytogenetics, Quantitative Genetics and Theory of Plant Breeding to postgraduate students. He supervised a large number of B.Sc. theses, 38 M.Sc. theses and 41 Ph.D. theses, six of these for foreign students.

Professor Borojević was not only a successful teacher and prolific researcher, he was also an enthusiastic extension officer, working on wheat production improvement at numerous agricultural combines and with technical services from all parts of the country, making his extensive knowledge and experience available to generations of agronomists.

Slavko Borojević was the recipient of high military decorations and peacetime awards: Certificate of Service 1941–1945, Medal of Partisan Star 3rd order (1945), Medal of Valour (1945), Medal of Brotherhood and Unity 1st order (1946), Medal of Merit to the People 2nd order (1948), Medal of Work 1st order (1961), October Award of the City of Novi Sad (1961 and 1971), 7th July Award of SR Serbia (1967), Vojvodina Liberation Award (1974), Gold Medal Mihajlo Pupin (1974), Award of the Antifascist Council of National Liberation of Yugoslavia (1975) and Medal of the Republic with Golden Wreath (1982).

As a great worker and humanist, Professor Borojević will remain a role model for generations of researchers and agronomists, and will be remembered as a resolute fighter for a better future.

S. DENČIĆ

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